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Presented for filing is a new provisional-to-utility patent application of:

Applicant: PAUL F. WORLEY AND ANTHONY LANAHAN
Title: IMMEDIATE EARLY GENES AND METHODS OF USE
THEREFOR

Enclosed are the following papers, including all those required to receive a filing date under 37 CFR §1.53(b):

	<u>Pages</u>
Specification	115
Claims	6
Abstract	1
Declaration (unsigned)	2
Small Entity Statement	[to be filed at a later date]

Under 35 USC §119(e)(1), this application claims the benefit of prior U.S. provisional application 60/074,518, filed 2/12/98 and 60/074,135, filed 2/6/98.

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BOX PATENT APPLICATION

February 5, 1999

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Basic filing fee	\$ 380.00
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Multiple dependent claims	0.00
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A check for the filing fee is enclosed. Please apply any other required fees or any credits to deposit account 06-1050, referencing the attorney docket number shown above.

If this application is found to be INCOMPLETE, or if a telephone conference would otherwise be helpful, please call the undersigned at 619/678-5070.

Kindly acknowledge receipt of this application by returning the enclosed postcard.

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Respectfully submitted,



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Enclosures

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APPLICATION
FOR
UNITED STATES LETTERS PATENT

TITLE: IMMEDIATE EARLY GENES AND METHODS OF USE
THEREFOR

APPLICANT: PAUL F. WORLEY AND ANTHONY LANAHAN

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M.E. Augustine

M.E. Augustine

IMMEDIATE EARLY GENES AND METHODS OF USE THEREFOR

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Related Applications

This application claims priority to U.S. provisional application nos. 60/074,518, filed February 12, 1998 and 60/074,135, filed February 6, 1998, both of which are incorporated herein by reference.

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Statement as to Federally Sponsored Research

Funding for the work described herein was provided by the federal government, which may have certain rights in the invention.

BACKGROUND

15 1. Technical Field

The present invention generally relates to gene expression and more specifically to immediate early genes in the brain and polypeptides encoded by such immediate early genes.

20 2. Background Information

An immediate early gene (IEG) is a gene whose expression is rapidly increased immediately following a stimulus. For example, genes expressed by neurons that exhibit a rapid increase in expression immediately following neuronal stimulation are neuronal IEGs. Such neuronal IEGs have been found to encode a wide variety of polypeptides including transcription factors, cytoskeletal polypeptides, growth factors, and metabolic enzymes as well as polypeptides involved in signal transduction. The identification of neuronal IEGs and the polypeptides they encode provides important information about the function of neurons in, for example, learning, memory, synaptic transmission, tolerance, and neuronal plasticity.

SUMMARY

The present invention involves methods and materials related to IEGs. Specifically, the invention provides isolated IEG nucleic acid sequences, cells that contain isolated IEG nucleic acid, substantially pure polypeptides encoded by IEG nucleic acid, and antibodies having specific binding affinity for a polypeptide encoded by IEG nucleic acid. In addition, the invention provides cDNA libraries enriched for IEG cDNAs, isolated nucleic acid derived from such cDNA libraries, and methods for treating conditions related to a deficiency in a neuron's IEG responsiveness to a stimulus.

In one aspect, the invention features an isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The isolated nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The hybridization conditions can be moderately or highly stringent hybridization conditions.

In another embodiment, the invention features an isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least five amino acids in length. The amino acid sequence contains at least three different amino acid residues, and is identical to a contiguous portion of sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features an isolated nucleic acid having a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

In another aspect, the invention features a substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

In another embodiment, the invention features a substantially pure polypeptide having an amino acid sequence as set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features a substantially pure polypeptide having an amino acid sequence at least 60 percent identical to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another embodiment of the invention features a substantially pure polypeptide having an amino acid sequence at least five amino acids in length. The amino acid sequence contains at least three different amino acid residues, and is identical to a contiguous stretch of sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62.

Another aspect of the invention features a host cell (e.g., a eukaryotic or prokaryotic cell) containing an isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The isolated nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another aspect of the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity for an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the
5 sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another aspect of the invention features a cDNA library having a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent (e.g., at least about 20 or 10 25 percent) of the clones have cDNA derived from immediate early genes (e.g., immediate early genes responsive to a maximal electroconvulsive seizure). The cDNA library can be a subtracted cDNA library. For example, the subtracted cDNA library can be the IEG-Reg or IEG-Lg cDNA library.

Another aspect of the invention features an isolated nucleic acid derived from a cDNA library. The cDNA library has a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent of the clones have cDNA derived from immediate early genes. The isolated nucleic acid can have a nucleic acid sequence of an immediate early gene.

Another aspect of the invention features a method of obtaining immediate early gene nucleic acid. The method includes providing a cDNA library having a plurality of clones with each clone having a cDNA insert. In addition, at least about 15 percent of the clones have cDNA derived from immediate early genes. The method also includes contacting at least a portion of the cDNA library with a probe containing at least one nucleic acid having a nucleic acid sequence derived from an immediate early gene, and selecting a member of the plurality of clones based on the hybridization of the at least one nucleic acid to the member under hybridization conditions.
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Another aspect of the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The

method includes administering a nucleic acid to the animal such that the effect of the deficiency is minimized. The nucleic acid has at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. In addition, the nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The deficiency can include a reduced level of expression of an immediate early gene. In addition, the stimulus can influence learning or memory. For example, the stimulus can include a maximal electroconvulsive seizure.

In another embodiment, the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering a therapeutically effective amount of a substantially pure polypeptide to the animal such that the effect of the deficiency is minimized. The polypeptide contains an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features a method of treating an animal (e.g., human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering an effective amount of cells to the animal such that the effect of the deficiency is minimized. The cells contain a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. In addition, the nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a

sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Another embodiment of the invention features a method of treating an animal (e.g.,
5 human) having a deficiency in a neuron's immediate early gene responsiveness to a stimulus. The method includes administering a therapeutically effective of antibodies to the animal such that the effect of the deficiency is minimized. The antibodies have specific binding affinity for an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic
10 acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The deficiency can include an elevated level of expression of an immediate early gene.

Another aspect of the invention features a method of identifying a compound that modulates immediate early gene expression. The method includes contacting a test compound with an immediate early gene nucleic acid, and determining whether the test compound effects the expression of the immediate early gene nucleic acid. The presence of an effect indicates that the test compound is a compound that modulates immediate early gene expression. The immediate early gene nucleic acid can contain a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60. The effect can be a reduction or increase in the expression of the immediate early gene nucleic acid.

In another embodiment, the invention features a method of identifying a compound that modulates immediate early gene polypeptide activity. The method includes contacting a test compound with an immediate early gene polypeptide, and determining whether the test compound effects the activity of the immediate early gene polypeptide. The presence of an effect indicates that the test compound is a compound that modulates immediate early gene

polypeptide activity. The immediate early gene polypeptide can contain an amino acid sequence encoded by a nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base. The nucleic acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second nucleic acid under

5 hybridization conditions. The second nucleic acid has a sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60.

Alternatively, the immediate early gene polypeptide can contain an amino acid sequence as set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62. The effect can be a reduction or
10 increase in the activity of the immediate early gene polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.
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DETAILED DESCRIPTION

The present invention provides methods and materials related to IEGs. Specifically, the invention provides isolated IEG nucleic acid, cells that contain isolated IEG nucleic acid, substantially pure polypeptides encoded by IEG nucleic acid, and antibodies having specific binding affinity for a polypeptide encoded by IEG nucleic acid. In addition, the invention provides cDNA libraries enriched for IEG cDNAs, isolated nucleic acid derived from such
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cDNA libraries, and methods for treating conditions related to a deficiency in a neuron's IEG responsiveness to a stimulus.

The present invention is based on the discovery of nucleic acid clones for many different neuronal IEGs. Specifically, nucleic acid clones for different neuronal IEGs were isolated and identified based on the ability of each IEG to rapidly increase expression upon seizure induction by a maximal electroconvulsive seizure (MECS) method (Cole *et al.*, *J. Neurochem.* 55:1920-1927 (1990)). It is important to note that MECS induction can be considered a model to study long-term plasticity relevant to learning and memory since it is known that a single MECS can produce extremely robust and long lived potentiation of synaptic contacts in the hippocampus and block spatial learning (Barnes *et al.*, *J. Neurosci.* 14:5793-5806 (1994)). Thus, MECS-responsive IEGs can influence neuronal activities involved in brain functions such as learning and memory. Moreover, the isolation and identification of IEG nucleic acid not only provides research scientists with information about neuronal activity and gene regulation but also provides methods and materials that can be used to manipulate brain function.

Each isolated IEG nucleic acid described herein can be used to produce a polypeptide. In addition, each IEG nucleic acid can be used to identify cells that are responsive to MECS induction. For example, an IEG nucleic acid can be labeled and used as a probe for *in situ* hybridization analysis. Clearly, having the ability to identify MECS-responsive cells provides one with the ability to isolate or monitor specific brain regions that are involved in learning. Further, any of the isolated partial IEG nucleic acid sequences can be used to obtain a full-length clone that encodes an IEG polypeptide. For example, a fragment from an isolated IEG nucleic acid can be radioactively labeled and used to screen a library such that a full-length clone is obtained.

Cells containing isolated IEG nucleic acid can be used to maintain or propagate the isolated IEG nucleic acid. In addition, such cells can be used to produce large quantities of polypeptides that are encoded by isolated IEG nucleic acid. Further, cells containing isolated IEG nucleic acid can be used to generate virus particles containing the isolated IEG nucleic acid. Such virus particles can be used *in vitro* or *in vivo* to provide other cells with the isolated IEG

nucleic acid. The polypeptides encoded by IEG nucleic acid can be used as immunogens to produce antibodies. Such antibodies can be used to identify MECS-responsive cells, monitor the level of polypeptide expression following MECS induction, and isolate polypeptides directly from animal tissue.

5 cDNA libraries enriched for IEG cDNAs can be used to isolate novel IEG cDNA.

Clearly, the isolation of novel IEG cDNAs is important to further the understanding of brain function. In addition, isolated nucleic acid derived from such cDNA libraries can be used to produce polypeptides as well as identify cells that are responsive to a stimulus such as MECS induction.

10 It is important to note that isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, and anti-IEG polypeptide antibodies can be used to treat conditions associated with a deficiency in a neuron's ability to express IEGs in response to a stimulus such as MECS. A condition associated with a deficiency in a neuron's IEG responsiveness to a stimulus is any physiological condition characterized as having a lack of a normal level of responsiveness. For example, when a deficiency in a neuron's responsiveness to MECS is characterized as a non- or under-expression of a particular IEG polypeptide by that neuron, the organism having the condition can be treated with isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, or substantially pure IEG polypeptides such that the effect of the deficiency is minimized. Alternatively, when a deficiency in a neuron's responsiveness to MECS is characterized as an over-expression of a particular IEG polypeptide by that neuron, the organism having the condition can be treated with anti-IEG polypeptide antibodies or the anti-sense strand of an isolated IEG nucleic acid such that the effect of the deficiency is minimized.

15 In addition, isolated IEG nucleic acid, cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, and anti-IEG polypeptide antibodies can be used to identify pharmaceutical compounds that can be used to treat diseases such as epilepsy, age-dependent memory decline, stroke, and drug addiction. For example, a compound that modulates IEG nucleic acid expression or IEG polypeptide activity can be identified by contacting a test

compound with either the IEG nucleic acid or polypeptide, and determining whether the test compound effects expression or activity.

The term “nucleic acid” as used herein encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

The term “isolated” as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term “isolated” also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid.

Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In

addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or 5 gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

Any isolated nucleic acid having a nucleic acid sequence as set forth in SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 28, 29, 31, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 is within the 10 scope of the invention. For convenience, these nucleic acid sequences will be referred to collectively as the IEG nucleic acid group. In addition, any isolated nucleic acid having a nucleic acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent identical) to a sequence set forth in the IEG nucleic acid group is within the 15 scope of the invention. For the purpose of this invention, the percent identity between a sequence set forth in the IEG nucleic acid group (designated a template sequence) and any other nucleic acid sequence is calculated as follows. First, the two nucleic acid sequences are aligned using the MEGALIGN® (DNASTAR, Madison, WI, 1997) sequence alignment software following the Jotun Heim algorithm with the default settings. Second, the number of matched positions between the two aligned nucleic acid sequences is determined. A matched position refers to a position in which identical bases occur at the same position as aligned by the 20 MEGALIGN® sequence alignment software. Third, the number of matched positions is divided by the total number of bases in the template sequence, and the resulting value multiplied by 100 to obtain the percent identity. If the obtained percent identity is greater than or equal to about 60 percent for a particular nucleic acid sequence, then that particular nucleic acid sequence is a 25 nucleic acid sequence at least about 60 percent identical to a sequence set forth in the IEG nucleic acid group.

Any isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99

percent identical) to the sequence set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, or 62 is within the scope of the invention. For convenience, the amino acid sequences set forth in SEQ ID NO:11, 21, 27, 30, 32, 36, 38, 48, 61, and 62 will be referred to collectively as the IEG amino acid group. For the purpose of this invention, the percent identity between a sequence set forth 5 in the IEG amino acid group (designated a template sequence) and any other amino acid sequence is calculated as follows. First, the two amino acid sequences are aligned using the MEGALIGN® (DNASTAR, Madison, WI, 1997) sequence alignment software following the Jotun Heim algorithm with the default settings. Second, the number of matched positions between the two aligned amino acid sequences is determined. A matched position refers to a 10 position in which identical residues occur at the same position as aligned by the MEGALIGN® sequence alignment software. Third, the number of matched positions is divided by the total number of amino acid residues in the template sequence, and the resulting value multiplied by 100 to obtain the percent identity. If the obtained percent identity is greater than or equal to about 60 percent for a particular amino acid sequence, then that particular amino acid sequence 15 is an amino acid sequence at least about 60 percent identical to a sequence set forth in the IEG amino acid group.

Any isolated nucleic acid having a nucleic acid sequence that encodes an amino acid sequence at least five amino acids in length also is within the scope of the invention provided the encoded amino acid sequence has at least three different amino acid residues, and is identical to a contiguous portion of sequence set forth in a sequence within the IEG amino acid group.

Further, any isolated nucleic acid having at least one adenine base, at least one guanine base, at least one cytosine base, and at least one thymine or uracil base is within the scope of the invention provided the isolated nucleic acid is at least about 12 bases in length (e.g., at least about 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or 60 bases in length), and hybridizes, under 20 hybridization conditions, to the sense or antisense strand of a nucleic acid having a sequence as set forth in the IEG nucleic acid group. The hybridization conditions can be moderately or highly stringent hybridization conditions.

For the purpose of this invention, moderately stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH7.4), 5X SSC, 5X Denharts solution, 50 µg/ml denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/ml probe (>5x10⁷ cpm/µg), while the washes are performed at about 50°C with a wash solution containing 2X SSC and 0.1% SDS.

Highly stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH7.4), 5X SSC, 5X Denharts solution, 50 µg/ml denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/ml probe (>5x10⁷ cpm/µg), while the washes are performed at about 65°C with a wash solution containing 0.2X SSC and 0.1% SDS.

Nucleic acid within the scope of the invention can be identified and obtained using any method including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, PCR can be used to obtain a nucleic acid having a nucleic acid sequence at least about 60 percent identical (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent identical) to a sequence set forth in the IEG nucleic acid group. PCR refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond are used to design oligonucleotide primers that are identical or similar in sequence to opposite strands of a potential template to be amplified. Using PCR, a nucleic acid sequence can be amplified from RNA or DNA. For example, a nucleic acid sequence can be isolated by PCR amplification from total cellular RNA, total genomic DNA, and cDNA as well as from bacteriophage sequences, plasmid sequences, viral sequences, and the like. When using RNA as a source of template, reverse transcriptase can be used to synthesize complimentary DNA strands.

Nucleic acid within the scope of the invention also can be obtained by mutagenesis. For example, a nucleic acid sequence set forth in the IEG nucleic acid group can be mutated using common molecular cloning techniques (e.g., site-directed mutageneses). Possible mutations

include, without limitation, deletions, insertions, and base substitutions, as well as combinations of deletions, insertions, and base substitutions.

In addition, nucleic acid and amino acid databases (e.g., GenBank[®]) can be used to identify and obtain a nucleic acid within the scope of the invention. For example, any nucleic acid sequence having some homology to a sequence set forth in the IEG nucleic acid group, or any amino acid sequence having some homology to a sequence set forth in the IEG amino acid group can be used as a query to search GenBank[®].

Further, nucleic acid hybridization techniques can be used to identify and obtain a nucleic acid within the scope of the invention. Briefly, any nucleic acid having some homology to a sequence set forth in the IEG nucleic acid group, or fragment thereof, can be used as a probe to identify a similar nucleic acid by hybridization under conditions of moderate to high stringency. Such similar nucleic acid then can be isolated, sequenced, and analyzed to determine whether they are within the scope of the invention as described herein.

Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The probe can be labeled with a radioisotope such as ³²P, an enzyme, digoxigenin, or by biotinylation. The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring harbor Laboratory, Plainview, NY. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in the IEG amino acid group. can be used to identify a nucleic acid identical to or similar to a nucleic acid sequence set forth in the IEG nucleic acid group. In addition, probes longer or shorter than 20 nucleotides can be used.

Any cell containing an isolated nucleic acid within the scope of the invention is itself within the scope of the invention. This includes, without limitation, prokaryotic and eukaryotic cells. It is noted that cells containing an isolated nucleic acid of the invention are not required to express the isolated nucleic acid. In addition, the isolated nucleic acid can be integrated into the

genome of the cell or maintained in an episomal state. In other words, cells can be stably or transiently transfected with an isolated nucleic acid of the invention.

Any method can be used to introduce an isolated nucleic acid into a cell. In fact, many methods for introducing nucleic acid into a cell, whether *in vivo* or *in vitro*, are well known to those skilled in the art. For example, calcium phosphate precipitation, electroporation, heat shock, lipofection, microinjection, and viral-mediated nucleic acid transfer are common methods that can be used to introduce nucleic acid into a cell. In addition, naked DNA can be delivered directly to cells *in vivo* as described elsewhere (U.S. Patent Number 5,580,859 and U.S. Patent Number 5,589,466 including continuations thereof). Further, nucleic acid can be introduced into cells by generating transgenic animals.

Transgenic animals can be aquatic animals (such as fish, sharks, dolphin, and the like), farm animals (such as pigs, goats, sheep, cows, horses, rabbits, and the like), rodents (such as rats, guinea pigs, and mice), non-human primates (such as baboon, monkeys, and chimpanzees), and domestic animals (such as dogs and cats). Several techniques known in the art can be used to introduce nucleic acid into animals to produce the founder lines of transgenic animals. Such techniques include, without limitation, pronuclear microinjection (U.S. Patent No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci., USA*, 82:6148-6152 (1985)); gene transfection into embryonic stem cells (Gossler A *et al.*, *Proc. Natl. Acad. Sci. USA* 83:9065-9069 (1986)); gene targeting into embryonic stem cells (Thompson *et al.*, *Cell*, 56:313-321 (1989)); nuclear transfer of somatic nuclei (Schnieke AE *et al.*, *Science* 278:2130-2133 (1997)); and electroporation of embryos.

For a review of techniques that can be used to generate and assess transgenic animals, skilled artisans can consult Gordon (*Intl. Rev. Cytol.*, 115:171-229 (1989)), and may obtain additional guidance from, for example: Hogan *et al.*, "Manipulating the Mouse Embryo" Cold Spring Harbor Press, Cold Spring Harbor, NY (1986); Krimpenfort *et al.*, *Bio/Technology*, 9:844-847 (1991); Palmiter *et al.*, *Cell*, 41:343-345 (1985); Kraemer *et al.*, "Genetic Manipulation of the Early Mammalian Embryo" Cold Spring Harbor Press, Cold Spring Harbor, NY (1985); Hammer *et al.*, *Nature*, 315:680-683 (1985); Pursel *et al.*, *Science*, 244:1281-1288

(1986); Wagner *et al.*, U.S. Patent No. 5,175,385; and Krimpenfort *et al.*, U.S. Patent No. 5,175,384.

Any method can be used to identify cells that contain an isolated nucleic acid within the scope of the invention. For example, PCR and nucleic acid hybridization techniques such as
5 Northern and Southern analysis can be used. In some cases, immunohistochemistry and biochemical techniques can be used to determine if a cell contains a particular nucleic acid by detecting the expression of a polypeptide encoded by that particular nucleic acid. For example, detection of polypeptide X-immunoreactivity after introduction of an isolated nucleic acid containing a cDNA that encodes polypeptide X into a cell that does not normally express
10 polypeptide X can indicate that that cell not only contains the introduced nucleic acid but also expresses the encoded polypeptide X from that introduced nucleic acid. In this case, the detection of any enzymatic activities of polypeptide X also can indicate that that cell contains the introduced nucleic acid and expresses the encoded polypeptide X from that introduced nucleic acid.
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In addition, any method can be used to force a cell to express an encoded amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, constructing a nucleic acid such that a regulatory element drives the expression of a nucleic acid sequence that encodes a polypeptide. Typically, regulatory elements are DNA sequences that regulate the expression of other DNA sequences at the level of transcription. Such regulatory elements include, without limitation, promoters, enhancers, and the like. Further, any methods can be used to identifying cells that express an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, immunocytochemistry, Northern analysis, and RT-PCR.
20

The term "substantially pure" as used herein with reference to a polypeptide means the polypeptide is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, a substantially pure polypeptide is any polypeptide that is removed from its natural environment and is at least 60 percent free, preferably 75 percent free, and most preferably 90 percent free from other components with which it is naturally
25

associated. Typically, a substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel.

Any substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention. In addition, any substantially pure polypeptide having an amino acid sequence at least about 60 percent (e.g., at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent) identical to a sequence set forth in the IEG amino acid group is within the scope of the invention. The percent identity between particular amino acid sequences is determined as described herein.

Any method can be used to obtain a substantially pure polypeptide. For example, one skilled in the art can use common polypeptide purification techniques such as affinity chromatography and HPLC as well as polypeptide synthesis techniques. In addition, any material can be used as a source to obtain a substantially pure polypeptide. For example, tissue from wild-type or transgenic animals can be used as a source material. In addition, tissue culture cells engineered to overexpress a particular polypeptide of interest can be used to obtain substantially pure polypeptide. Further, a polypeptide within the scope of the invention can be "engineered" to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag[®] tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that could be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

The term "antibody" as used herein refers to intact antibodies as well as antibody fragments that retain some ability to selectively bind an epitope. Such fragments include, without limitation, Fab, F(ab')2, and Fv antibody fragments. The term "epitope" refers to an antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules (e.g., amino acid or sugar residues) and usually have specific three dimensional structural characteristics as well as specific charge characteristics.

Any antibody having specific binding affinity for an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention. Thus, any monoclonal or polyclonal antibody having specific binding affinity for an amino acid sequence set forth in the IEG amino acid group is within the scope of the invention. Such antibodies can be used in immunoassays in liquid phase or bound to a solid phase. For example, the antibodies of the invention can be used in competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays include the radioimmunoassay (RIA) and the sandwich (immunometric) assay.

Antibodies within the scope of the invention can be prepared using any method. For example, any substantially pure polypeptide provided herein, or fragment thereof, can be used as an immunogen to elicit an immune response in an animal such that specific antibodies are produced. Thus, an intact full-length polypeptide or fragments containing small peptides can be used as an immunizing antigen. In addition, the immunogen used to immunize an animal can be chemically synthesized or derived from translated cDNA. Further, the immunogen can be conjugated to a carrier polypeptide, if desired. Commonly used carriers that are chemically coupled to an immunizing polypeptide include, without limitation, keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, e.g., Green *et al.*, *Production of Polyclonal Antisera*, in IMMUNOCHEMICAL PROTOCOLS (Manson, ed.), pages 1-5 (Humana Press 1992) and Coligan *et al.*, *Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters*, in CURRENT PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992). In addition, those of skill in the art will know of various techniques common in the immunology arts for purification and concentration of polyclonal antibodies, as well as monoclonal antibodies (Coligan, *et al.*, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994).

The preparation of monoclonal antibodies also is well-known to those skilled in the art. See, e.g., Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726 (Cold Spring

Harbor Pub. 1988). Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by analyzing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, *Purification of Immunoglobulin G (IgG)*, in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (Humana Press 1992).

In addition, methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication *in vitro* can be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally replenished by mammalian serum such as fetal calf serum, or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, and bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells (e.g., syngeneic mice) to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

The antibodies within the scope of the invention also can be made using non-human primates. General techniques for raising therapeutically useful antibodies in baboons can be

found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer* 46:310 (1990).

Alternatively, the antibodies can be “humanized” monoclonal antibodies. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions (CDRs) from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions when treating humans. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l. Acad. Sci. USA* 86:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522 (1986); Riechmann *et al.*, *Nature* 332:323 (1988); Verhoeyen *et al.*, *Science* 239:1534 (1988); Carter *et al.*, *Proc. Nat'l. Acad. Sci. USA* 89:4285 (1992); Sandhu, *Crit. Rev. Biotech.* 12:437 (1992); and Singer *et al.*, *J. Immunol.* 150:2844 (1993).

Antibodies of the present invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 119 (1991) and Winter *et al.*, *Ann. Rev. Immunol.* 12: 433 (1994). Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been “engineered” to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens and can be used to produce human antibody-secreting

hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13 (1994); Lonberg *et al.*, *Nature* 368:856 (1994); and Taylor *et al.*, *Int. Immunol.* 6:579 (1994).

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of an intact antibody or by the expression of a nucleic acid encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of intact antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulphhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg (U.S. Patent Nos. 4,036,945 and 4,331,647). See also Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959); Edelman *et al.*, METHODS IN ENZYMOLOGY, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used provided the fragments retain some ability to selectively bind its epitope.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l. Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, e.g., Sandhu, *supra*. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding polypeptides (sFv) are prepared by constructing a nucleic acid construct encoding the V_H and V_L domains connected by an oligonucleotide. This nucleic acid construct is inserted into an expression vector, which is subsequently introduced

into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271-77 (1993); and Sandhu, *supra*.

Another form of an antibody fragment is a peptide coding for a single CDR. CDR peptides (“minimal recognition units”) can be obtained by constructing nucleic acid constructs that encode the CDR of an antibody of interest. Such constructs are prepared, for example, by using PCR to synthesize the variable region from RNA of antibody-producing cells. See, e.g., Larrick *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 106 (1991).

It is also possible to use anti-idiotype technology to produce monoclonal antibodies that mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the “image” of the epitope bound by the first monoclonal antibody. Such anti-idiotypic monoclonal antibodies can be used to inhibit the activity of the polypeptide containing the original epitope.

The invention also provides cDNA libraries enriched for IEGs. As described herein, such cDNA libraries contain an increased frequency of cDNAs derived from IEGs. Specifically, about 15 percent (e.g., about 20 or 25 percent) of the cDNA clones within the cDNA libraries provided herein are derived from IEGs.

A cDNA library within the scope of the invention can be prepared from any tissue containing cells that express an IEG (e.g., hippocampus tissue). Again, an IEG is a gene whose expression is rapidly increased immediately following a stimulus. The stimulus can be electrical or chemical in nature. For example, cells can be treated with electric shock or chemicals such as kainate. Briefly, cDNA libraries are prepared from the hippocampus of control animals (e.g., rats) as well as from animals that receive a stimulus (e.g., multiple MECS) using, for example, a

phage vector lambda ZAP II (Stratagene). A subtracted library is then prepared using *in vitro* mRNA prepared from a control library and subsequent solution phase hybridization with cDNA prepared from a stimulated library. The control *in vitro* mRNA can be tagged with biotin to permit its removal from solution using avidin beads (Lanahan *et al.*, *Mol. Cell. Biol.* 12:3919-3929 (1992)). cDNA that remains after removal of mRNA/cDNA hybrids can be recloned into, for example, a lambda ZAPII phage vector. Several rounds of subtraction (e.g., two, three, four, or five rounds) can be used to increase the frequency of IEGs. The subtracted library then can be plated and duplicate phage lifts screened with a radiolabeled cDNA probe. Any probe can be used provided it contains at least one nucleic acid sequence derived from an IEG. For example, a probe can be prepared from mRNA obtained from the hippocampus of a stimulated animal. In addition, the mRNA used to make a probe can be subjected to subtractive hybridization such that IEG sequences are enriched. In general, conventional cDNA libraries contain IEGs at a frequency of <1:30,000 cDNAs. For the cDNA libraries enriched for IEGs, however, about 1 in 5 genes can be induced by a stimulus such as MECS. This represents an about 1000 to 10,000 fold enrichment in brain IEGs.

An animal (e.g., human) having a deficiency in a neuron's IEG responsiveness to a stimulus (e.g., a stimulus that influences learning or memory) can be treated using the methods and materials described herein. A stimulus that influences learning or memory can be a multiple MECS treatment. A deficiency in a neuron's IEG responsiveness to a stimulus means the level of IEG responsiveness is not normal. Such deficiencies can be identified by stimulating a sample of cells and measuring the levels of IEG expression. If the levels are not similar to the levels normally observed in a similar tissue sample, then there is a deficiency. It is noted that increased IEG expression as well as decreased IEG expression can be classified as a deficiency provided the levels are not normal.

A deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a nucleic acid of the invention to the animal such that the effect of the deficiency is minimized. The administration can be an *in vivo*, *in vitro*, or *ex vivo* administration as described herein. For example, an *in vivo* administration can involve administering a viral vector

to the hippocampal region of an animal, while an *ex vivo* administration can involve extracting cells from an animal, transfecting the cells with the nucleic acid in tissue culture, and then introducing the transfected cells back into the same animal.

In addition, a deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a therapeutically effective amount cells containing isolated IEG nucleic acid, substantially pure IEG polypeptides, anti-IEG polypeptide antibodies, or combinations thereof. A therapeutically effective amount is any amount that minimizes the effect of the deficiency while not causing significant toxicity to the animal. Such an amount can be determined by assessing the clinical symptoms associated with the deficiency before and after administering a fixed amount of cells, polypeptides, or antibodies. In addition, the effective amount administered to an animal can be adjusted according to the animal's response and desired outcomes. Significant toxicity can vary for each particular patient and depends on multiple factors including, without limitation, the patient's physical and mental state, age, and tolerance to pain. The cells, polypeptides, or antibodies can be administered to any part of the animal's body including, without limitation, brain, spinal cord, blood stream, muscle tissue, skin, peritoneal cavity, and the like. Thus, these therapeutic agents can be administered by injection (e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, intracavity, or transdermal injection) or by gradual perfusion over time.

Preparations for administration can include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Other vehicles for administration include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles containing fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Further, a deficiency in a neuron's IEG responsiveness to a stimulus can be treated by administering a therapeutically effective amount of a compound that directly interferes with the translation of IEG nucleic acid. For example, antisense nucleic acid or ribozymes could be used to bind to IEG mRNA or to cleave it. Antisense RNA or DNA molecules bind specifically with a targeted RNA message, interrupting the expression of the mRNA product. The antisense binds to the messenger RNA forming a double stranded molecule that cannot be translated by the cell. Typically, an antisense oligonucleotide is about 15-25 nucleotides in length. In addition, chemically reactive groups, such as iron-linked ethylenediaminetetraacetic acid (EDTA-Fe), can be attached to an antisense oligonucleotide, causing cleavage of the mRNA at the site of hybridization. These and other uses of antisense methods to inhibit the translation of nucleic acid are well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289 (1988)).

An oligonucleotide also can be used to stall transcription winding around double-helical DNA and forming a three-strand helix (Maher, *et al.*, *Antisense Res. and Dev.*, 1:227 (1991) and Helene, *Anticancer Drug Design*, 6:569 (1991)).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. By modifying nucleic acid sequences that encode ribozymes, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030 (1988)). There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, *Nature*, 334:585 (1988)) and “hammerhead”-type. Tetrahymena-type ribozymes recognize sequences that are four bases in length, while “hammerhead”-type ribozymes recognize sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, “hammerhead”-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species. In addition, 18-based recognition sequences are preferable to shorter recognition sequences. These and other uses of antisense methods to inhibit the *in vivo* translation of nucleic acid are well known in the art (DeMesmaeker *et al.*, *Curr. Opin. Struct.*

Biol. 5:343-355 (1995); Gewirtz *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A.*, 93:3161-3163 (1996); and Stein, *Chem. Biol.* 3:319-323 (1996)).

Delivery of nucleic acid, antisense, triplex agents, and ribozymes can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system.

5 Various viral vectors that can be utilized for gene therapy include adenoviruses, herpesviruses, vaccinia viruses, and retroviruses. A retroviral vector can be a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. In addition, a nucleic acid sequence of interest along with another nucleic acid sequence that encodes a ligand for a receptor on a specific target cell can be inserted into a viral vector to produce a vector that is target specific. For example, retroviral vectors can be made target specific by inserting a nucleic acid sequence that encodes an antibody that binds a specific target antigen. Those of skill in the art can readily ascertain without undue experimentation specific nucleic acid sequences that can be inserted into a retroviral genome to allow target specific delivery of the retroviral vector containing the nucleic acid of the invention.

A colloidal dispersion system can be used to target the delivery of the nucleic acid of the invention. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles that are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV) that range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. Thus, nucleic acid, intact virions, polypeptides, and antibodies can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley *et al.*, *Trends Biochem. Sci.*, 6:77 (1981)). In addition to mammalian cells, liposomes have been used to deliver nucleic acid to plants, yeast, and bacteria. In order for a liposome to

be an efficient nucleic acid transfer vehicle, the following characteristics should be present: (1) encapsulation of the nucleic acid of interest at high efficiency while not compromising its biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of the nucleic acid (Mannino *et al.*, *Biotechniques*, 6:682 (1988)).

The composition of a liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids also can be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. In general, the compounds bound to the surface of the targeted delivery system will be ligands and receptors that allow the targeted delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest that will bind to another compound, such as a receptor or antibody.

Compounds that modulate IEG expression can be identified by contacting a test compound with an IEG nucleic acid, and determining whether the test compound effects expression. Likewise, compounds that modulate IEG polypeptide activity can be identified by contacting a test compound with an IEG polypeptide, and determining whether the test

compound effects polypeptide activity. Contacting includes in solution and in solid phase, or in a cell. Any type of compound can be used as a test compound including, without limitation, peptides, peptidomimetics, polypeptides, chemical compounds, and biologic agents. In addition, the test compound can be a combinatorial library for screening a plurality of compounds.

5 Compounds identified using the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, *et al.*, *Bio/Technology*, 3:1008-1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, *et al.*, *Proc. Nat'l. Acad. Sci. USA*, 80:278 (1983),

10 oligonucleotide ligation assays (OLAs; Landegren, *et al.*, *Science*, 241:1077 (1988), and the like.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 - Construction of subtracted cDNA libraries

15 The mRNA used to prepare the cDNA libraries was obtained from the hippocampus of adult rats (male or female). Briefly, the hippocampus was dissected from naive or stimulated rats, and rapidly frozen in liquid nitrogen. The stimulation protocol used to stimulate the rats was as follows. Rats were injected intraperitoneally with 50 mg of the protein synthesis inhibitor cycloheximide (50 mg/ml stock in 50% ethanol) per kilogram of body weight 15 minutes prior to initiating repetitions of maximal electroconvulsive seizure (MECS). MECS was induced by passage of a constant current signal by means of an ECT unit (Ugo, Basil). The current signal lasted one second with a frequency of 100 Hz. Each pulse lasted 0.5 milliseconds, and the current was 90 milliamperes. This stimulus caused brief loss of consciousness and a tonic-clonic seizure lasting 30 seconds to one minute. MECS was administered about every 15 minutes for a total of 13 administrations over the course of 2.5 to 3 hours. Thirty (30) minutes after the last MECS, the rats were sacrificed by decapitation.

To collect total RNA, the tissue was homogenized in 4M guanidinium thiocyanate using a polytron and then centrifuged through a CsCl cushion. To isolate polyA⁺ RNA, the resulting total RNA was chromatographed on oligo(dT) columns using a commercial oligo(dT) resin and purification protocol (Fastback, Invitrogen). About 50 naive (control) and 50 stimulated rats
5 were used to generate the polyA⁺ mRNA needed to make the cDNA libraries and perform the Northern blot analysis.

A nonsubtracted cDNA library was made using polyA⁺ RNA isolated from rats subjected to MECS. Briefly, cDNA was synthesized and cloned directionally into the Lambda ZAP vector yielding a library containing 3.6×10^6 recombinants. This library was designated the 3 hr
10 MECS/CHX library. Differential screening of the 3 hr MECS/CHX library with control and stimulated rat hippocampal cDNA probes yielded several novel IEGs. Analysis of these IEGs revealed that they were relatively abundant.

The 3 hr MECS/CHX library was used as starting material for preparing a subtracted cDNA library highly enriched for IEGs. A subtracted cDNA library highly enriched for IEGs can allow for the detection of lower abundance novel IEGs. To make a subtracted cDNA library,
15 DNA template was prepared from the 3 hr MECS/CHX library as follows.

The 3 hr MECS/CHX library was amplified and plated on 15 cm NZCYM agarose plates at a density of about 50,000 phage/plate. A total of 1.85×10^6 phage were plated on a total of 37 plates. The plates were overlaid with Suspension Media (SM) and the phage particles eluted by swirling at 4°C overnight. The lysate was collected, and chloroform added to a final concentration of 5%. The lysate was clarified by centrifugation, and the phage containing supernatant collected and stored at 4°C. A 300 ml aliquot of the lysate was treated with RNaseA (final concentration of 1 µg/µl) and DNase I (final concentration of 1 µg/µl) for three hours at 37°C. Polyethylene glycol (PEG 6000) was added to a concentration of 10%, and NaCl added to a concentration of 1 M. After mixing well, the lysate was stored at 4°C overnight to allow phage particles to precipitate. Phage particles were pelleted by centrifugation, resuspended in 20 ml of SM, and stored at 4°C. Phage particles were lysed by adding EDTA to a concentration of 10 mM and SDS to a concentration of 0.2% followed by a 20 minute incubation at 68°C. Polypeptides
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were removed by two extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by two extractions with chloroform/isoamyl alcohol (24:1). The phage DNA contained within 40 ml of lysate was precipitated by adding 1/10th volume of 3M NaOAc (pH 5.2) followed by the addition of 2 volumes of 100% ethanol. After mixing, the solution was incubated at -20°C overnight. DNA was pelleted by centrifugation, resuspended in 10 mM Tris, 1 mM EDTA pH 7.5 (TE), and reprecipitated overnight. After this second precipitation, the DNA was pelleted by centrifugation and resuspended in 12 ml of 10 mM Tris (pH 7.5), 5 mM EDTA, 300 mM NaCl. To remove residual RNA, RNase A (final concentration of 50 µg/ml) was added followed by incubation at 37°C for 1 hour. To remove RNase A, SDS (final concentration of 0.5%) and then Proteinase K (final concentration of 50 µg/ml) was added followed incubation at 37°C for 1 hour. The DNA lysate was extracted twice with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). After this extraction, the DNA lysate was dialyzed against 12 liters of TE for 2 days at 4°C. The 300 ml aliquot of phage lysate yielded 7254 µg of phage DNA. This phage DNA was then used to prepare *in vitro* polyA⁺ RNA (cRNA).

To prepare *in vitro* cRNA, the phage DNA template was linearized at the 3' end of the cDNA insert using the restriction enzyme XhoI. Briefly, 1 mg of phage DNA was digested with 1000 U of XhoI for three hours at 37°C. After the three hour incubation, an additional 1000 U of XhoI was added and the 37°C incubation continued an additional three hours. XhoI was removed by adding SDS to 0.5% and Proteinase K to 50 µg/ml followed by incubation at 37°C for one hour. Polypeptides were removed by three extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). The DNA was precipitated with 1/10th volume 3M NaOAc (pH 5.2) and 2 volumes 100% ethanol. The DNA was pelleted by centrifugation and resuspended in 500 µl TE (1.58 mg/ml final DNA concentration).

This linearized DNA was used as template to prepare *in vitro* cRNA from the sense strand of the cDNA inserts. This cRNA is representative of the initial *in vivo* population of RNA in the MECS/cycloheximide treated rat hippocampus. Forty (40) µg of DNA template was

incubated with 40 mM Tris (pH 7.5), 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 1 U/ μ l RNasin, 500 μ M ATP, 500 μ M CTP, 500 μ M GTP, 500 μ M UTP, and 2 U/ μ l T3 RNA polymerase in a final volume of 300 μ l for two hours at 40°C. After two hours, an additional 2 U/ μ l of T3 RNA polymerase was added, and the reaction incubated for an additional
5 two hours at 37°C for a total time of four hours. The DNA template was removed by adding DNaseI (2 U/ μ g of template) and incubating the mixture at 37°C for an hour. Polypeptides were removed by two extractions with phenol/chloroform/isoamyl alcohol (50:48:2) followed by one extraction with chloroform/isoamyl alcohol (24:1). The cRNA was precipitated at 20°C with one half volume 7.5 M NH₄OAc and 2 volumes 100% ethanol. The cRNA was pelleted and
10 resuspended in TE. The cRNA was chromatographed on sephadex G-50 columns (NICK columns; Pharmacia) to remove free nucleotides and the concentration of cRNA determined by UV absorbance at 260 A. Thirty (30) μ g of DNA template yielded 68.6 μ g of cRNA. The cRNA was either stored frozen at -20°C or precipitated with 1/10th volume 2 M KOAc (pH 5) and 2 volumes 100% ethanol. The 68.6 μ g of cRNA was further purified using oligo(dT)
15 column chromatography to select polyA⁺ cRNA. The cRNA was bound to oligo(dT) under high salt conditions, rinsed with low salt conditions, and eluted with TE (pH 7.5). This eluted cRNA was again passed over an oligo(dT) column under high salt conditions, rinsed with low salt conditions, and the polyA⁺ cRNA eluted with TE (pH 7.5). The two passes on oligo(dT) cellulose yielded 34.2 μ g of polyA⁺ cRNA. This polyA⁺ cRNA was then used as template for
20 synthesis of first strand cDNA that was then subtracted against control brain and liver polyA⁺ RNA.

Two cDNA synthesis reactions were performed to prepare first strand cDNA from the polyA⁺ cRNA. One involved using 2 μ g of cRNA with a small amount of ³²P-dCTP to allow for the analysis of subtraction efficiency, and the other involved using 5 μ g of cRNA with no
25 radioactive dNTPs. The radioactive cDNA synthesis reaction was as follows. First, 2 μ l cRNA (1 μ g/ μ l in TE), 1 μ l Xho(dT) primer (1.4 μ g/ μ l), and 8 μ l water was combined, and the mixture was incubated at 70°C for ten minutes, quickly spun, and placed on ice. Second, 1 μ l RNasin (40 U/ μ l), 5 μ l 5X Reaction Buffer (BRL), 2.5 μ l 0.1M DTT, 1.5 μ l dNTP mix, and 2 μ l ³²P

dCTP (3000 Ci/mmole) was added, and the mixture was incubated at room temperature for ten minutes. The dNTP mix contained 10 mM of each dATP, dGTP, and dTTP as well as 5 mM of methyl dCTP. After incubation, 2 μ l of Superscript/MMLV RT mix (1:1) was added, and the mixture (25 μ l total volume) was incubated at room temperature for five minutes followed by a 5 90 minute incubation at 40°C. The nonradioactive cDNA synthesis reaction was as follows.

First, 5 μ l cRNA (1 μ g/ μ l in TE), 2 μ l Xho(dT) primer (1.4 μ g/ μ l), and 3 μ l water was combined, and the mixture was incubated at 70°C for ten minutes, quickly spun, and placed on ice. Second, 1 μ l RNasin (40 U/ μ l), 5 μ l 5X Reaction Buffer (BRL), 2.5 μ l 0.1M DTT, and 1.5 μ l dNTP mix was added, and the mixture was incubated at room temperature for ten minutes.

10 After incubation, 5 μ l of Superscript/MMLV RT mix (1:1) was added, and the mixture (25 μ l total volume) was incubated at room temperature for five minutes followed by a 90 minute incubation at 40°C.

15 After completion, 3.2 μ l of 0.5 M EDTA (pH 8.0) was added to the radioactive reaction, and then the radioactive and nonradioactive reactions were combined. For subtractive hybridizations, it was necessary to remove the cRNA template by alkaline hydrolysis. This was done by adding 25 μ l of TE (pH 7.5) and 5.8 μ l of 2 M NaOH. This resulted in a 20 mM final concentration of EDTA and a 138 mM final concentration of NaOH. The mixture was heated for 30 minutes at 68 to 70°C, and then 12.2 μ l of 1 M Tris (pH 7.5) and 5.8 μ l of 2 N HCl was added to neutralize the reaction. The final volume was 100 μ l of which 2 μ l was removed and counted to determine the percent incorporation of 32 P-dCTP into cDNA. This analysis revealed that 7000 ng of cRNA was converted to 2598 ng of first strand cDNA. This first strand cDNA was subtracted against adult rat brain and liver polyA⁺ RNA.

20 For the subtractive hybridizations, the first strand cDNA was chromatographed on a sephadex G-50 column (NICK, Pharmacia) to remove unincorporated dNTPs, especially the unincorporated 32 P-dCTP in order to allow the efficiency of subtraction to be followed. After the cDNA was eluted from the NICK column, it was mixed with 60 μ g of adult rat brain polyA⁺ 25 RNA that was coupled to biotin (2X Bio RNA). The cDNA and polyA⁺ RNA mixture was precipitated by adding 1/10th volume 3M NaOAc (pH 5.2) and 2 volumes 100% ethanol. This

mixture then was pelleted and resuspended in 20 µl TE (pH 7.5) and 20 µl 2X Subtraction Hybridization Buffer (100 mM Hepes (pH 7.6), 0.4% SDS, 4 mM EDTA, 1 M NaCl). The resuspended cDNA and polyA⁺ RNA mixture was then incubated at 95 °C for two minutes, quickly spun, and submerged in a 60 °C water bath for 48 hours to allow hybrids to form

5 between the cDNA and biotinylated polyA⁺ RNA (BioRNA).

The cDNA/BioRNA complexes were removed as follows. First, 40 µl 1X Subtraction Hybridization Buffer lacking SDS and 20 µl Streptavidin (1 mg/ml) was added, and the resulting mixture incubated at room temperature for ten minutes. After incubation, the cDNA/BioRNA complexes were removed by extraction with phenol/chloroform/isoamyl alcohol. The phenol phase was back-extracted with 1X Subtraction Hybridization Buffer lacking SDS, and the aqueous phases pooled. Once pooled, 20 µl Streptavidin (1 mg/ml) was added, and the resulting mixture incubated at room temperature for ten minutes. After incubation, remaining cDNA/BioRNA complexes were removed by extraction with phenol/chloroform/isoamyl alcohol. The phenol phase was back-extracted with 1X Subtraction Hybridization Buffer lacking SDS, and the aqueous phases pooled. The pooled aqueous phases (about 400 µl) were extracted with chloroform/isoamyl alcohol. At this point, an aliquot of the aqueous phase was counted to determine the amount of cDNA remaining. Results revealed that 78% of the starting cDNA was removed with 22% remaining (572 ng).

To perform a second round of subtraction, the aqueous phase (about 400 µl) containing the non-hybridizing first strand cDNA was mixed with 30 µg of adult rat brain polyA⁺ RNA coupled to biotin and 30 µg of adult rat liver polyA⁺ RNA coupled to biotin. The cDNA and biotinylated polyA⁺ RNA was co-precipitated and hybridized as described for the first round. In addition, the cDNA/BioRNA complexes were removed as described above, and the percentage of non-hybridizing cDNA remaining was determined. Results revealed that two rounds of subtraction removed 87.5% of the starting cDNA with 12.5% of the starting cDNA remaining.

25 A third round of subtraction similar to the second round was performed using the remaining cDNA. Analysis of the remaining cDNA revealed that the three rounds of subtraction had removed 90% of the starting cDNA leaving 10% of the starting cDNA (255 ng).

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The remaining single stranded cDNA was used to synthesize double stranded cDNA for the subtracted cDNA library. First, the single stranded cDNA (300 µl) was alkali treated to remove any remaining RNA as follows. The final concentration of EDTA was adjusted to 20 mM by addition of 13 µl of 0.5M EDTA, and then 20 µl of 2M NaOH (120 mM final concentration) was added. This mixture was incubated at 68°C for 30 minutes and then neutralized by adding 40 µl 1 M Tris (pH 7.5) and 20 µl 2 N HCl. The cDNA was precipitated by adding 10 µl glycogen (10 mg/ml), 1/10th volume 3M NaOAc (pH 5.2), and 2 volumes ethanol. The cDNA then was pelleted, resuspended in 100 µl of TE (pH 7.5), and purified on a sephadex G-50 column (NICK, Pharmacia). The purified cDNA was re-precipitated using glycogen, pelleted, and resuspended in TE (pH 7.5) as described. Second, 50 µl resuspended cDNA (single stranded, subtracted cDNA), 20 µl 5X Sequenase Buffer, and 13 µl water was combined, and the mixture incubated at 65°C for five minutes, 37°C for ten minutes, and room temperature for 30 minutes. After incubation, 5 µl dNTP mix, 5 µl 0.1 M DTT, 2 µl Sequenase (13 U/µl), and 2 µl Klenow (5 U/µl) was added, and the mixture (100 µl final volume) incubated at 37°C for one hour. The dNTP mix contained 10 mM dATP, 10 mM dCTP, 10 mM dGTP, and 10 mM dTTP. The reaction was terminated by adding 3 µl of 0.5 M EDTA (pH 8.0) followed by two extractions with phenol/chloroform/isoamyl alcohol and a final extraction with chloroform/isoamyl alcohol. The double stranded cDNA was ethanol precipitated, pelleted by centrifugation, and resuspended in 86 µl TE (pH 7.5).

The double stranded cDNA was then restriction digested as follows. Eighty-six (86) µl cDNA, 10 µl 10X EcoRI Reaction Buffer (NEB), 2 µl EcoRI (20 U/µl), and 2 µl XhoI (20 U/µl) was combined, and the mixture (100 µl final volume) incubated at 37°C for one hour. After this incubation, an additional 2 µl EcoRI (20 U/µl) and 2 µl XhoI (20 U/µl) was added, and the mixture again incubated at 37°C for one hour. After digestion, the reaction was extracted twice with phenol/chloroform/isoamyl alcohol followed by one chloroform/isoamyl alcohol extraction. The digested cDNA was precipitated with ethanol, pelleted by centrifugation, and resuspended in 40 µl of 10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM NaCl, and 20 µl loading buffer. The cDNA was divided into two aliquots, and each aliquot was size-fractionated on a 1 ml BioGel A-

5 50m column. The columns were rinsed with 10 mM Tris (pH 7.5), 1 mM EDTA, and 100 mM NaCl, with 50 µl fractions being collected. One column was run to select for only relatively long cDNAs while the other was run to select for all cDNAs. These separate pools were then extracted twice with phenol/chloroform/isoamyl alcohol followed by one chloroform/isoamyl
10 alcohol extraction. The cDNA was precipitated by adding 5 µl yeast tRNA (1 µg/µl) and 2 volumes of 100% ethanol. The cDNA was pelleted by centrifugation and directionally cloned into lambda phage UniZAP as follows. For the regular cDNAs (all sizes), 4 µl water, 2 µl 5X Ligase Buffer (BRL), 2 µl UniZAP (500 ng/µl), and 2 µl T4 DNA Ligase (10 U/µl) was added to the pelleted cDNA, and the mixture (10 µl final volume) incubated at 14°C overnight. For the
15 large cDNAs, 2 µl water, 1 µl 5X Ligase Buffer (BRL), 1 µl UniZAP (500 ng/µl), and 1 µl T4 DNA Ligase (10 U/µl) was added to the pelleted cDNA, and the mixture (5 µl final volume) incubated at 14°C overnight. The ligated cDNA was then packaged using packing extracts (Stratagene) and titered on XL1-Blue MRF cells. The subtracted 3 hr MECS/CHX cDNA library containing large cDNAs (designated IEG-Lg cDNA library) had 239,000 recombinants, and the subtracted 3 hr MECS/CHX cDNA library containing regular cDNAs (designated IEG-Reg cDNA library) had 4,992,000 recombinants. A portion of each library was rescued as pBluescript plasmid, and the cDNA inserts analyzed. Of 46 plasmids analyzed from the IEG-Lg cDNA library, all contained cDNA inserts with the average insert size being 1.36 kilobases. Of 44 plasmids analyzed from the IEG-Reg cDNA library, 43 contained cDNA inserts with the
20 average insert size being 0.9 kilobases.

25 Duplicate southern blots containing cDNA from the 44 plasmids analyzed from the IEG-Reg cDNA library were probed with control and stimulated subtracted ³²P-oligolabeled cDNA from rat hippocampus. Eleven of the 44 cDNA inserts gave a clear differential signal that was stronger with the 3 hour MECS/CHX cDNA probe than with the control cDNA probe. This result indicates that 1 in 4 of the clones in the IEG-Reg cDNA library is derived from an IEG.

Example 2 - Preparation of subtracted cDNA probes

Subtracted cDNA was prepared using exactly the same protocol described in example 1 with the exception that rather than *in vitro* cRNA being used as the template for cDNA synthesis, polyA⁺ RNA derived from control rat hippocampi or rat hippocampi from rats treated with the 3 hour MECS protocol was used. After first strand cDNA synthesis, the RNA template was denatured by alkaline hydrolysis, and the free nucleotides removed by chromatography on sephadex G-50 (NICK, Pharmacia). The cDNA was precipitated using 1/10th volume 3M NaOAc (pH 5.2), 2 µl glycogen (20 mg/ml), and 2 volumes ethanol, pelleted by centrifugation, and resuspended in TE (pH 7.5). The final concentration was 25 ng/µl. The single strand of cDNA was labeled to high specific activity ($2\text{-}4 \times 10^9 \text{ cpm}/\mu\text{g}$) by oligolabelling (Pharmacia) with ³²P dCTP (3000 Ci/mmol). Free nucleotides were removed by chromatography on sephadex G-50 (NICK column, Pharmacia), and the purified ³²P-labeled subtracted cDNA used to probe the subtracted cDNA libraries.

Example 3 - Screen subtracted libraries

The IEG-Reg and IEG-Lg cDNA libraries were plated on NZCYM agarose plates at a density of 500-800 plaques/plate. Duplicate nitrocellulose filter lifts were prepared from each plate using standard techniques. The filters were prehybridized overnight at 68°C in 5X SSPE (pH 7.4), 10% dextran sulfate, 0.2% SDS, 5X Denhardt's Solution, and 50 µg/ml boiled, sonicated salmon sperm DNA. The first lift from each plate was then hybridized with $4 \times 10^6 \text{ cpm}/\text{ml}$ of the control subtracted cDNA probe and the second lift with $4 \times 10^6 \text{ cpm}/\text{ml}$ of the 3 hour MECS stimulated subtracted cDNA probe. Hybridization was done in freshly prepared 5X SSPE (pH 7.4), 10% dextran sulfate, 0.2% SDS, 5X Denhardt's Solution, and 100 µg/ml boiled, sonicated salmon sperm DNA at 68°C for three days. Filters were washed twice at room temperature for 30 minutes in 2X SSC/0.2% SDS, twice at 60°C for two hours in 0.5X SSC/0.2% SDS, and then dried and exposed to X-Ray film for one to seven days. Clones

exhibiting greater hybridization signals with the stimulated cDNA probe than those observed with the control cDNA probe were picked for further analysis.

The putative neuronal IEGs were analyzed by reverse northern analysis and northern analysis to confirm that they were true differentially hybridizing cDNAs. The nucleotide sequence from the ends of these cDNAs was determined, and those sequences not matching the sequences of known genes were used to obtain full-length cDNAs from cDNA libraries.

Example 4 - Construction of a cDNA library enriched for near full-length IEG cDNAs

Since the initial isolates for all of the IEGs represented small cDNAs derived from the 3' regions of the corresponding RNA, it was necessary to rescreen other libraries to obtain full-length or near full-length cDNAs. For this purpose, a cDNA library enriched for neuronal IEGs with very long inserts was prepared from 3 hour MECS/CHX polyA⁺ RNA isolated from rat hippocampi. This RNA was already relatively enriched for neuronal IEGs since the MECS/CHX stimulus produces a large induction of IEG expression. Further, the cDNA was synthesized in the presence of methylmercuric hydroxide to eliminate RNA secondary structure allowing for the synthesis of long cDNAs using Superscript II Reverse Transcriptase (BRL).

The basic protocol used to synthesize cDNA was as follows. First, RNA secondary structure was denatured with methylmercuric hydroxide which forms adducts with imino groups of uridine and guanosine in the RNA and disrupts Watson-Crick base pairing. Briefly, 22 µl polyA⁺ RNA (0.5 µg/µl in either 10 mM Tris/1 mM EDTA (pH 7.0) or water) was incubate at 65°C for five minutes and then cooled to room temperature over five minutes. Once cooled, 2.2 µl 100 mM CH₃HgOH (90 µl depc'd water plus 10 µl 1 M CH₃HgOH) was added, and the mixture incubated at room temperature for one minute. After incubation, 4.4 µl 700 mM 2-mercaptoethanol (190 µl depc'd water plus 10 µl 14 M 2-mercaptoethanol) was added, and the mixture (final volume 28.6 µl) incubated at room temperature for five minutes.

Second, the first strand of cDNA was synthesized as follows. The volume of the denatured RNA mixture was adjusted by adding 26.4 µl water such that the concentration of RNA was 0.2 µg/µl. In the radioactive reaction, 5 µl (1 µg) polyA⁺ RNA, 2 µl 10X Strand 1

Buffer (Stratagene), 1.2 μ l Strand 1 dNTP mix (Stratagene), 0.8 μ l Xho/dT linker primer (1.4 μ g/ μ l), 5 μ l water, 3 μ l dCTP³² 3000 Ci/mmol (NEN), and 1 μ l RNase Block (Stratagene) was combined, and the mixture (final volume 18 μ l) incubated at room temperature for ten minutes to allow the primer to anneal to the RNA. In the nonradioactive reaction, 25 μ l (5 μ g) polyA⁺ RNA, 5 μ l 10X Strand 1 Buffer (Stratagene), 3 μ l Strand 1 dNTP mix (Stratagene), 2 μ l Xho/dT linker primer (1.4 μ g/ μ l), 9 μ l water, and 1 μ l RNase Block (Stratagene) was combined, and the mixture (final volume 45 μ l) incubated at room temperature for ten minutes to allow the primer to anneal to the RNA. After the room temperature incubation, 2 μ l and 5 μ l of reverse transcriptase mix (4 μ l Superscript II (BRL 200 U/ μ l) plus 1 μ l MMLV RT (Stratagene)) was added to the radioactive and nonradioactive reactions, respectively. The reactions then were incubated at 40°C for one hour and placed on ice. Two μ l of cDNA was removed from the radioactive reaction and added to 18 μ l T₁₀E₁ and 2 μ l 0.5M EDTA. Two (2) μ l of this mixture then was applied to a PEI strip to determine the percent incorporation and quantity of cDNA synthesized, while 18 μ l was mixed with sample buffer and ran on a gel to assay cDNA quality.

Third, the second strand of cDNA was synthesized as follows. Both the radioactive and nonradioactive reactions were kept on ice to prevent "snapback" cDNA synthesis. For the radioactive reaction (18 μ l), 10 μ l 10X Second Strand cDNA Buffer, 3 μ l Second Strand dNTP mix, 62.5 μ l water, 1 μ l RNaseH (1.5 U/ μ l), and 5.5 μ l DNA Polymerase I (9 U/ μ l) was added, and the mixture (100 μ l final volume) incubated at 16°C for 2.5 hours. For the nonradioactive reaction (50 μ l), 20 μ l 10X Second Strand cDNA Buffer, 6 μ l Second Strand dNTP mix, 111 μ l water, 2 μ l RNaseH (1.5 U/ μ l), and 11 μ l DNA Polymerase I (9 U/ μ l) was added, and the mixture (200 μ l final volume) incubated at 16°C for 2.5 hours. Four (4) μ l of cDNA was removed from the radioactive reaction and added to 18 μ l T₁₀E₁ and 2 μ l 0.5M EDTA. Two μ l of this mixture then was applied to a PEI strip to determine the percent incorporation and quantity of cDNA synthesized, while 18 μ l was mixed with sample buffer and ran on a gel to assay cDNA quality.

The cDNA from both the radioactive and nonradioactive reactions were extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl

alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 39.5 µl water. To blunt the cDNA ends, 5 µl 10X T4 DNA Polymerase Buffer (NEB), 2.5 µl dNTP mix (2.5 mM each dNTP), and 3 µl T4 DNA Polymerase (3 U/µl) was added to the 39.5 µl of cDNA, and the mixture (50 µl final volume) incubated at 16°C for 30 minutes. After incubation, 350 µl TE (pH 7.5) was added, and the mixture (400 µl final volume) extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 17 µl water.

EcoRI/NotI adaptors were ligated to the cDNA, allowing for the quick identification of artifactual cDNAs generated by the ligation of two independent cDNAs prior to ligation into the lambda phage vector. To ligate the EcoRI/NotI adaptors to the cDNA, 3 µl 10X Ligase Buffer, 4 µl EcoRI/NotI Adaptors (1 µg/µl), 3 µl 10 mM ATP, and 3 µl T4 DNA Ligase (400 U/µl) was added to the 17 µl cDNA, and the mixture (30 µl final volume) incubated at 10°C overnight. After the overnight incubation, 1 µl T4 DNA Ligase and 1 µl 10 mM ATP was added, and the mixture (32 µl final volume) again incubated at 10°C overnight. After this second overnight incubation, 270 µl TE (pH 7.5) was added and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 30 µl water.

To kinase the cDNA ends, 4 µl 10X T4 Polynucleotide Kinase Buffer, 4 µl 10 mM ATP, and 2 µl T4 Polynucleotide Kinase (10 U/µl) was added to the 30 µl of cDNA, and the mixture (40 µl final volume) incubated at 37°C for 30 minutes. After incubation, 2 µl T4 Polynucleotide Kinase was added, and the mixture (42 µl final volume) incubated at 37°C for 30 minutes. After this second 30 minute incubation, 170 µl TE (pH 7.5) was added, and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 85 µl water.

To digest the 3' cDNA ends with XhoI, 10 μ l 10X NEB Buffer #2 and 5 μ l XhoI (20 U/ μ l) was added to the 85 μ l of cDNA, and the mixture (100 μ l final volume) incubated at 37°C for 45 minutes. After incubation, 3 μ l XhoI (40 U/ μ l) was added, and the mixture (103 μ l final volume) again incubated at 37°C for 45 minutes. After this second incubation, 120 μ l TE (pH 5 7.5) was added, and the mixture extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated with 100% ethanol, pelleted by centrifugation, and resuspended in 20 μ l 10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM NaCl, and 5 μ l loading buffer. This resuspended cDNA then 10 was size-fractionated on a 1 ml BioGel A-50m column to select large cDNAs. The column was rinsed with 10 mM Tris (pH 7.5), 1 mM EDTA, and 100 mM NaCl. Thirty-six (36) fractions (50 μ l/fraction) were collected. Aliquots from individual fractions were electrophoreses on 1% agarose to identify fractions containing cDNAs longer than 2 kilobases. Such fractions were pooled, and the resulting mixture of pooled fractions was extracted twice with phenol/chloroform/isoamyl alcohol followed by one extraction with chloroform/isoamyl alcohol. After extraction, the cDNA was precipitated by adding 2 μ l glycogen (20 mg/ml) and 2 volumes 15 100% ethanol, pelleted by centrifugation, and resuspended in 5 μ l water.

To directionally clone the cDNA into UniZAP, 2 μ l UniZAP (500 ng/ μ l), 1 μ l 10X T4 DNA Ligase Buffer, 1 μ l 10 mM ATP, and 1 μ l T4 DNA Ligase (4000 U/ μ l) was added to the 5 μ l of cDNA, and the mixture (10 μ l final volume) incubated at 12°C overnight. After 20 incubation, the cDNA was packaged into phage particles. To package the cDNA, the ligation reaction (10 μ l final volume) was divided into two packaging reactions with each containing 5 μ l of ligation reaction together with a packaging extract (Stratagene). This mixture was incubated at 22°C for 2 hours. After incubation, the two reaction mixtures were pooled and the library titered on IL1-Blue MRF cells.

This 3 hr MECS/CHX library (designated IEG-FL 3 hr MECS/CHX cDNA library) had a titer of 4.4×10^6 primary phage. The library was amplified and used to isolate full length cDNAs derived from novel neuronal IEGs. The relative abundance of near full length neuronal IEG cDNAs in this library was substantially higher than the levels experienced using other cDNA

libraries. In a single cDNA library screen, full length cDNAs for four different novel IEGs were obtained. Three of the four IEG cDNAs were derived from mRNAs of 4 kilobases, while one was derived from an mRNA of 3 kilobases.

5 The nucleic acid sequencing of the IEG cDNAs was performed at Johns Hopkins School of Medicine and at Applied Biosciences, Inc., CA using the Sanger method with fluorescent dye termination.

Northern blot analysis was performed both to confirm that the cloned cDNAs represent tissue mRNA that is rapidly induced by brain activation and to assess the size of the mRNA transcript. The latter is essential information for the identification of authentic full length clones.

10 Either 20-25 µg of total RNA or 2 µg of polyA⁺ RNA was sized by denaturing agarose gel chromatography and transferred to nitrocellulose. Blots were then hybridized with [³²P]labeled cDNAs. Labelling was done using the random primer method (Pharmacia).

In addition, *in situ* hybridization was performed both to confirm that the cloned cDNAs represent tissue mRNA that is rapidly induced by brain activation and to confirm that the mRNA was induced in activated neurons. *In situ* hybridization was performed as described previously (Andreasson and Worley, *Neuroscience* 69: 781-796 (1995)).

Example 5 - IEG nucleic acid

The following clones were identified as being IEG nucleic acid as described in Example

20 3. In addition, certain clones were identified by chip-hybridization between PCR fragments generated from rat hippocampus ESTs and ³²P-dCTP-labeled cDNA derived from polyA⁺ RNA of rat hippocampus from MECS treated animals and controls.

One IEG nucleic acid clone was designated A003. The first library screen produced a fragment (A003-1-1) of 1.6 kilobases (kb) with a polyA sequence at the 3'-end. A second round of screening was performed using a probe prepared from the 5'-end of A003-1-1. This screen produced two clones: A003-1 (2.8 kb) and A003-2 (1.3 kb). The fragments from the secondary screen were sequenced from both ends. These fragments formed a contig at their 3'-end with the A003-1-1 fragment. The following two nucleic acid sequences are within the A003 clone: 5'-

TTGCAGATCAGCACCTTTGATGATGCCTGCCAACAGTGGTAATGCTNACAGCAA
AGCACCACTTACGCTTTAGTGTGCTGGTTCATGGCTGGACATACACCAACCA
GCCTGACCCCACAGGAATGCCAAGTTGGCTGGAATGTAACCCAACCTAGTTCTGC
GCTTCGCTCCTCTCCAGTGCAAGGTGCTAAACACCCACTCACAAGCCTGCTGTCAA
5 GCTGCGACCTGGGGCTGGTAGAAAGGGCTGCCTCCTCCAGCAATAGAAGTTCA
TGAATTGAGGCTGGAGATAGGTCAAGACCACTGTGATAACTATAAAGACTGTAGC
AGCCACAAAGGAGACCCCCAAATAACTGGAGGCATGGCACTGACGTACCAGATGA
GGTTATTTGGAGCTGAAGGCTGCTCTGCTCTGGTAGCATCTTGTCCCT
TGGGACATGGTGANCCATACTGTCCACTGAGCTGGAGATGACAGTTGAATAAAA
10 AAAAAAAAAAAAAA-3' (SEQ ID NO:1) and 5'-CGGCTTAATTAACCCCTCACTAAA
GGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAAACTAGTGGATCC
CCCAGGCTGCAGGATTCTCGGGCCGATTAAGAACGCTGCTGATGTCCTTAGGCGAGG
ACATTAACCTCCAGTCTCTGACAGACTTGGACATCCAGAATAAGTTCTTTGTATAT
CAGAGCACAGAGCCCAGCTTAGCCTCTGATGGACCTCAGGAACCAAGAACGGAGGG
15 ACTTCCTAACATTCTAGAGATGGGACTCTAACTCTAGCTCTGTGTTAACGCCCTGAA
GTCCAGAAAGAAGTAGTTCTTGACATTCTAGTGCCAAGATCCAGCCTCTAACAGAGAA
CTCTGATGTCTAACAGAAAGTCTTCATAGTCTAGNCCAGTCACCAGTGAAGCTAAC
ACCTGAAAACATTAGATTCTCTGGAGCCAGGAATCCATCTCAAGTCTCTCATAAAG
20 CCCAAATGTCCCAGGAGAAGTTGACAATATAAAGCCGTATCTCGATGGACTTTGAA
GAAGCTCAGAAAAGGAGACCACCTGGTAGTCTGATCTAGGACTCTGGCTTGTGTTG
TCTCCAGGGACGTTACATGTATAAAAGAGGGACCTTCTGATGATTAGAACACTGG
GAECTCCACCTCCATCCTTGATGAAAGCTCAAATGTCCAGAAAGAGGGGCCTCTG
ATATTCTAGAGTAGGACCCCTCCAGCCTTGATGGTGTCCAGATGTCCAGAAAG
25 AGGGGCCTCTGTGATGTTCCAGACCTAGGGCCCTCCAGCCTTGATGGTGTCC
AGATGTCCAGAAAGAGGGACTCTGATGTTCCAGACCTAACAGACTCTAGCTCCAGC
CTTGATGAAGCTCAGATGTTAGAAAGGGGGCCTCCATGATGTTCTAGAACCCAGG
ACTCCACCTCTAGCCTTGATGGTGTCCAGATGTCCAGAAAGAGGGCTTCCATGAT
TTCTAGGACCAAGACTTACCTCCAGCCTCTATGCCTCCATGTCTCCAGTAAAGCTT

AGGTGTCCAGAAAAGAGCATTCTCAATGAATTATAGAACCGAGGACTCTTCTCCAG
CCTTGATGACGTTAGATGTCATAAAGAAGAACTTCCACAATGTACTAAAGCTAT
GAECTCCATCTCCATCCTTGATGAAAAGGGACTCCTCCACTCTGTTCCAGAACGCCT
AGCTCCACCTCTAATCTTGTGATGTCCAATTATCCAGAAAGAGGGGGCCTTAGA
5 ACAAAAGACTGTACTTTATTGATAAAAGCACAGATTCCAGAACGCACAGAAATCT
AGAAAGAGGGTCCTCCCTAACACCGCTCGAGCTAGAACCCCGGTGCAAGGGTCTGAA
ACTTAGACACCAGAAGACCGCTTGTCTACAACAAGTCTGCATTTCTAAATCTCC
AGGTGGCTGAT:CAGAAGGGTCCAGGAAGGTATGGGG-3' (SEQ ID NO:2).

Northern blot analysis using the 3'-end of A003 revealed the presence of two mRNA
transcripts. The more abundant transcript was 2.2 kilobases in length, while the less abundant
transcript was 4.8 kilobases in length. This analysis also revealed that the expression of A003
mRNA was marginally upregulated in response to the multiple MECS treatment. The multiple
MECS treatment involved the induction of multiple maximal electroconvulsive seizures
followed by the preparation of total RNA from rat hippocampus four hours post-seizure. This
multiple MECS treatment was designed to mimic ischemia.

Another IEG nucleic acid clone was designated A013. The first library screen produced
the clone designated A013-8. The 5'-end of A013-8 was used as a probe for the second round of
screening. This second screening produced two additional clones: A013-4 and A013-26. The
A013-8, A013-4, and A013-26 clones were sequenced using either the gene specific primer used
to generate the probe for the second round of library screening, or the T3 and T7 primers. Both
A013-4 and A013-26 made a contig on their 3'-ends with the A-013-8 clone. In addition, the
sequence from the 5'-ends of A013-4 and A013-26 revealed that they from contigs between each
other. Further, the sequence data from the 5'-ends of A013-4 and A013-26 revealed the presence
of an open reading frame of at least 720 basepairs (bp). Based on the combined length of the
20 obtained clones, the A013 clone is at least 3.0 kb in length. The following two nucleic acid
sequences are within the A013 clone: 5'-GGCACGAGATCACTCAGTGTCTCACTGAAC
CAAATCGTCATTTACAGAGAGATGCAAAGCTCAGCGAAGACATTTAGCTTTTT
AAAATGTATAATTCTGTGGCTACATATGCAAGTAGGGTCCCATTATGTTTTTTCA

TTAGTGGAAACTAACCTTTGTGCTGTAAATCAGTATTAGCTTATAGAATTAT
AAATGTATATTCTACTTCTGATCAAAGAACGTAGTCGGGTATTGGTTAGAAGITC
AAAGTGACACTGTATAGGGCTTCACGGTAATGGATTGTTAGCAAATCTAAGGA
CATACAGCCAATGATTATCTGAGGTTACTGGCTAACTGTTTCACTGAGTTACTCTG
5 CCTTTTGACATTATTCTTGTTCAGAATCCAGAGCTTCAGGAGCCCCAAATT
TTTTATWCCGTATATATATATATAAATATCCATAAGCCTGGGATTGTATG
CAATGCACTGCATCTATGTATTCTGATAGCATCTCATTGATTTGTTGAAATAGAA
AGAAAGATAGTATCCAAATGAGTTATCTTAACAGAAAGCTGAGTTAACTTTAT
TACCTATATAATAATTGATATTGCCAATTACCATTCTGAATTTCATATAGTATAAGTT
10 AGACATTGCTTAATCCCCTTAAATGTATTACATAGACATGAACACTCAAATTGCT
GGATTTTAAATATATCTGACATAATTTCATCTGTTACATTCAAGTTAGCTTGT
TAGCCCAGATTTCAGAATAGTAAAGGAGGAAAGGAACCGCATTCCAGGGAAACCT
CTGAGGCCAAGTCAGAGTCCAGAACTGTAAACACACAGGCCCTGCAAGCCAACATTA
GTCGTGAAATCCCTAACACGTCACTGGATTCTCTGTCAGCGCAAGTGTCAAGCTGC
CAAAGAATAGACTTACATGAAGAAGTGCACATGCTGGCAGGGCTGGCCGGCTC
CGGCCAGCAGACACTGCTAGATTGAATATTAAAGGTCGAGTTGACCTGTGGTAC
ACAGCTGTGCTGTGCTCAGCAACCTCAGAACTCTGAAAAAAACATAAAAAAG
AAAAAAAAAAAAAAAAMATGCACTGKYTCATTGTAATAGTGAATAGTAAAG
GAAAGAAAGGAAAACCAAAAGCTTGTCCATCACAGGTATGAGCTGCTATGATTCA
TGAAGAACATTCCATGGAGTATGTTAAACCTGTTATCTGAGAGGCTTAAA
AGCCAACCTAACTGTTAGGGCAACCGCGGTACAGACGTGGTCTCTGTGAGACTTC
CACCTGACCCAAGTTAACGTGGTACGAATGTTGTCATTAAATGTTAAGGACAGTC
TGCAATAATAAGTAAGTAGCCAGCGTGGTGCCAGCAGTGTGAGACCTGGCTGC
TCTATTGTACGCTTGGAAACACAATTATGCAACAGATGTCCAGATATGATTCTATT
25 TATGGAAAAAGTTATATGTTACAAATGGTTACCATCTTATATTAAATGACCTT
TTGACAGGTGTGCACTGTTGTCTCCAGTGAGCACATACCATGCGGATTTATATGT
ACATCAGTAGTGTGAATCCACTGGCACAGTGTGTAAATGCCAGATGTGGTGAGAT
TTTATCTGTATATGTGATCAGATAAAACTCCTGACAGAAACTGTAAGGRAACC

CAGCTGAATGGTTGACCTGGATGRCYKRKRTKGWTGGTTATGTTAAATGTATAT
TCTTTAATCAATGAATAAAGCATTAAAAATGGGAAAAAAAAACTCGTGC-3'
(SEQ ID NO:3) and 5'-TCTGCGGCCGCAGCATCCGAACAACAGGAACCTCCAGAA
5 GTTAGTCTTTGGAGATATAAGTGTGTTCAGCAGCAAGGAAGTCTGTCCAGCAC
ATACCTCAGCAGAGTAGACCCTGACGGCAAGAAGATTAAGCAAATTCAAGCAGCTGT
TTGAAGAGATACTGAGCAATAGTAGGCAACTAAAATGGCTGTCCGTGGTTATGC
TGGAAATAGTAACCCCATCATCACTGTCGTCTGTCTAACTCCATTGCCAACACCAT
GGAACACCTGAGTTACTGGACAACAACATTCTGGTAACAGCACGCTCATCACCAC
AGTCGAACTAGAGCGCTTGTAAATCTGCGCTCACTGCCCTGGATTCTGTGACTTT
10 ACAGCTGAGATGGCGAGAGTCCTGACCGACAGCAACCATGTGCCCTTGAGCGACT
GTCTCTTCTGGTCCACAATGCTTCAGTGATGCTCAAGTCATTAGACAACATGCCAAA
CGATGAGCACTGGAAGGCCCTGTCACGAAAGAGCTCCAGCCTCCGGTCTATCTAAT
GGCTTTGATGTTAAAGTGAAGACATGCTAAAGATTCTGAAACCCAGTATACCACT
TGAGAAGGGTTCACTTGGACAGCTACGTCACTTGTGTCTCAAGGGCTATTGGTTG
15 ATCTTATATTCCAGGCAGTATTGACCAAGGTTCTYAACCCMWTWTATTGATGA
ATGATATGATTGATACGTCGGTTCCGGATCTTAGTGACAACCGAAATGAAGATC
CATTGGTTTATTGGCATGGCGGTGCACAAAGCTCACTCTTGGCAATTGTTA
CACCCTGTGGGCACACAACCTCATTGCCATTGCTCGTCTCGTGGCTYTGACCTAA
AAGTGCTTGGAAAGTCACCSRAAGAAAGCATTGATTTGACCAAGGTGAACTAGCCC
20 GACCAGGAATGTGGRWYCCGTACATAACCTTCTGGAGCAGGTATTCCCTGGGGC
CTTGGTCAAGTCTGGCACG-3' (SEQ ID NO:4).

Northern blot analysis using a sequence from the A013 clone revealed the presence of a 3.2 kb mRNA transcript. In addition, this analysis revealed that the expression of the A013 mRNA was strongly upregulated in response to the multiple MECS treatment. Specifically, 25 A013 mRNA expression was induced 8.9 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

Table I. Fold induction of mRNA expression after multiple MECS treatment

Probe (rat cDNA)	Fold induction (normalized for the S26)
A013	8.9
L094	7.3
L100	17.2
L119	17.8
R113	7.0
R286	2.4

Another IEG nucleic acid clone was designated A020. The following nucleic acid sequence is within the A020 clone: 5'-TCAAACCNATCTCGGTATTCTTTGAT
TNATAAGGGATTKSCCGATKTCCGCNTATTGGTAAAAAWTGAGCTGATTAAACA
AAAATTAAACGCGAATTAAACAAAATATTAACGCTTACAATTGCCATTGCCATT
CAGGCTGCGCAAYTGTGGGAAGGGCNATCGGTGCAGGGCTTCGCTATTACGCCA
GCTGGCGAAAGGGGGATGTGCTGCAAGGCAGTTAACGTTGGTAACGCCAGGGTTT
CCCAGTCACGACGTTGAAAACGACGCCAGTGAATTGTAATACGACTCACTATAGG
GCGAATTGGGTACCGGGCCCCCTCGAGGTCGACGGTATCGATAAGCTGATATCG
AATTCCGGCACGAGCGAAGCCAGGGCCTGCACCTCCTAGGCAAGCGCTTACCACTG
AGCTAAATCCCCAACCCCTGTTTATTAAAGCAAACGAGATAACATAATTCARC
CATGATAATTAAAGATTATCTGAACCTTAAGGAAATGTATATACTAAGCTATTAT
AGTTTTATTTCCTAATTCACTGAGTCATAATACCTTACCTTGAGTCGTTACTACTTT
CTTGTTCTAAAAACTCTACTGCTAAATTACAATGTAAAAACATAGGGCTCGTAT
ATACTGTAGAGTGCTGTAGATGTCCTCGTCATCAACTATGCAATAACAGTCTGATCG
ACACATTTCAGGAKCGATCACTTTGGTGTGCTTAAATACTTCAAGCTTA
GGATGTGCAAAGCAGGAAGACTGTGGGTGTAAATGTTACTTATTCTTGAGAGTG
TTAGTAAGTCTTCDAAAATTGCTTTCTCTAAATTATCGTTAACTTAAATGATA
ATTATCTTGAGGTTAACAGAACAGCTATTGACAAACTAAAGTGACTTTAGGGCA
TTCTTGAGATCATAGTCTTATCTGGGGACTAAATGTCATTAGACCCTAATAGA

CTAACTTGTATGTTGTGGGGAAACGTTTCCTCTCATTCAAGGTAACGTGTTG
CTGCCTGTTACTGTGAGCATTCTAGAAAATGGCTAGGTTTTATAAGATTAA
AGACAATAGAAGTAGTTATATTATTAGTTCTGTTGGAATGTGATCCTGAAATT
ATTACTGAAAATTAGAATTTCGCTAACAGAACCTGACTCTCAGAGATGC
5 AGTGTAAATTGATAACCTCATCTTCCGAGAGTCAGAGCACAGGGCGGCAGTATGTG
AAGCTGCTTGCACTGACGCATTGATAAGTTGGCTACTGTAATGGTAAAAGGC
TCCTCAGGCACTGACTGCATTGGGTTCTCCGATGGGGATGATCCGTTCTCGTGGT
GCTGCTGGACTTATGCATTGGAGGTACTGCATGTATCTCCACACTGCTTGACATT
TTCTCTGATCTGTGTTGCACCAACTCATTAAAAGAAATATGCAGAAATATCTTCT
10 AATTCTGATCTCGCTGTATGACAGTTATAATATTAAACACTGGGTTGATCCACT
CTGTTACATTATCTTCTAACGCGTCAGAAAGGGACTAACTGAAATTATCTAGA
GGCTTGATCATTCAAAAATTAAATTCCCTGGATACTTAGGCAATATCTAAC
AACTTTTAATAAAATTAAATTTATTTACGTAAGCTAAATATACATGAATGTG
CTTTTAATAAAATTAAACAGTTACTTATTGCCAATTCAAATAAAAAAAAAA
15 AAAAAAAAAAAAAA-3' (SEQ ID NO:5). This clone is similar to GLGF-domain protein
Homer (accession # U92079).

Another IEG nucleic acid clone was designated A021. The following nucleic acid sequence is within the A021 clone: 5'-TTTTTTTTTTTTTTAARGGGRCCACCCCC
ACCGSGCTAAAGGCCAGGGCCCCCCCCTGGAGMCCCAGGGTTTGGCCCMCC
20 CCCTCACCAAATGGTCTGCCAATGACCCAGGTACTCACACATGTTCCAGGAGGAG
MCTGGGCCAGGATTGACCAGAGGGTATGGGAAGGGAAAGGGAGAAGAAATC
GACATTATTATTATTATTAAATGTTACAWTTCTTGTGTTCCAAGCC
CTGAATAGAAACAGATAGCATTAAAGGACTCTGTTCCCACCCCTCTGTCTCTC
TCCCCCACTGTGCTAACCTAGGATAACACTCTATTGTTCTAAAGTGA
25 TTTGTGGACTTGTGCCGTGAACTGCATTAAAAAGGTTCTGTTCAAAGATCGATT
GTCGTTCTGTGGGGACAGTGGCTCTAACAGAAATCTGCATTGTAGGAGAAGACAATG
AAAGACCCCTGGCCCTGTCTCTCAAAACTAACACTCTGTATGATTAAAAAAATT
CCATTACTTACTTGTGGTTACTTGATTGAGGAAGAAATATTCAACTTGTAT

Sequence 6

AAAGACTAGGTATCAGGGTTCTTGCAGTGGGAGTTGTATATATATCGTATTTGG
TATATCGTAGAAACTCAAGCTTATGCATCCGTATTGGGATATGTCATGACGTGC
AGTGAATTCGCTATTAGACCCTGGAGGCAAACGAGTTGACAAGGTTATGGCTC
CATGGGAATTCTAATTCTTCTGGGACCTTGTCCCCTTACAGTAATGGT
5 GAAATGGCCTAGGAGGGCTCTAGTCGAATTCTCCAGGCAGGACCACGTGCTCA
AAAAATCTTGTATAGTTAAATTTGAGGAGTATCTGCTCAGAACATCTGTG
GTGGTGTGTTGCGTTCTGTACTGTGTGACACAAGCCTACAGTATTGCA
CTAAGGAAAGCTGTTAGAGCTGCTGCTATGGAGGGAAAGAACATATTAAAAC
10 TTTCCCTCGGGGWTRWCWMGTTATGTWCTTGTCTGGCTTCCTACT
TTCCACTGAGTAGCATTGTAGAATAAAATGAATTAAGATCAGMWRWRWRMAAA
AAA-
3' (SEQ ID NO:6). This clone is similar to fra2.

Another IEG nucleic acid clone was designated A024. The following two nucleic acid sequences are within the A024 clone: 5'-TCAGGCCTNAGCAATCCTCNTTAANTTGA
15 NCCAAGNTTAACTCTGGGGCGAATTCCCTGTGNTTGCTTCTTCCCCATANTCCAG
GCCACAAAANGTTCTGTGANTCCGAGAATCGGCCACCATGCAGACCCACNGAG
AGGATTCAAATGTGTGAGAGTGAGTGAGTGTGAGTGCCTGCGTGTGCTTGTAT
GTGTGTTATAGATGTAGGACATTAAGTCCCTCTGACACAGGGAAAGATGTGAGAAG
20 GATGGCCTGACATCAGATGACAAGAGGTCTTATAGCACATCTGGCTTCCCTA
CCCAGAGAAGAGCCCCCTTGATACAAATCAGTGGATTTCATATGCTCAAAGGC
TTGATCTGTGAGTCACTCCAGTTGGACATAGGTCTGTGGCTTGAGAAAAG
GTACTTCAAAAGAGGGCTTCCAGAGCACAGCTCACAGCCAGCTGTAGGACCCA
25 CCCTCTCCTTATTGTGGAGGTGACTCACAGCAGACTGACAGTGGTCAGACTGAGC
TTCTGCTAAGGTGGTAGCCAACACTGGCATGTCTCGGTAGTGGTTGGCA
AATTCCGCAGGTCTCTCCCCAACCCCTGCCTCTGATGAATAAGACAATGAGTAC
AGTCCTTAATTCAAGGCTTGTGACTAGCTTACTACGGAACCGAAAATGGTCCCCT
TGTACAAGCCGAGCTGTATGGAATCACGGTAACAGACCCAGGTCTGTGGCACCT
GTTTGTTTTTTTTTTTTTAGCTCTACGGCATGCTTCCAAG

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GAACCAAAGGAGGGTCTCAGAGATGCCCAAACATCCAAAGTACACAAAGCTAAG
TAATCGATTGCTACTTATTGCACAGCTAGACACGGATTAAAGTCTATCTAAAGCT
TTGAAGCAAGCTTAGCTCTCAAAGGCCTAGCAGAGCCTGGCACCCCAGGATCCT
TCTGTAGGCTAATTCTTATCCAGCGCATATGGAGTATCCTTATTGCTAAAGAG
GATTCTGGCTCCTTAAGGAAGTTGATTCTGATTCAAGTCCTGTTCCCTGACT
TGCTCTGCCAGCCCTGCACCAGCTTTCGAAGTGCACATGCTTGTGTTAACTTCT
CCCAGTTTATTGGGCATAAAAGTTGTCCTTATTGTAAGCTGTTATAAAATAT
ATATTATATAAATATGACAAAGGAAAATGTTCAGATGTCTATTGTATAATTAC
TTGATCTACACAGTGAGGAAAAAAATGAATGTATTCTGTTTGAAGAGAATAATT
TTTTCTCTAGGGAGAGGAGAGGTTACAGTGTATATTGAAACCTCCTGAAGGT
GTGAAATTGTAATATTATCTAAGTAAATGTTAAGTAGTTGTTAAAAAGACTT
AATAAAATAAGCTTTCTGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
(SEQ ID NO:7) and 5'-GTGGCCCTGCTGCCGCATCATGGAGCGGATCCCCAGCG
CGCAACCACCTCCTACCTGCCTGCCAAAACGCCAGGGCTGGAGCACGGAGACCTG
TCAGGGATGGATTGCCCACATGTACCAAGTGTACAAGTCCAGGCAGGGAAATAAA
ACGGAGCGAGGACAGCAAGGAAACTTACAATTGCCGCACCGGTTGATTGAGAAAA
AGAGACGTGACCGGATTAACGAGTGCATTGCCAGCTGAAGGATCTCCTACCCGAA
CATCTCAAACTTACTACTTGGTCACTGGAGAAAGCAGTGGTCTCGAGCTGACG
CTGAAGCACGTGAAAGCATTGACAAACCTAATTGATCAGCAGCAGCAGAAAATCAT
GCCCTGCAGAGCGGTTACAAGCTGGTATCTGCGGAAAGAAATTGAGGCAG
GACAAGAAATGTTCTGCTCCGGTTCCAGACCTGTGCCCGGGAGGTACTCAGTACC
TGGCCAAGCATGAAAACACTAGGGACCTGAAGTCTCCAGCTGCTCACTCATCTCC
ACCGTGTGGTCTCTGAACCTGCAGGGTAGTGCTCCAGGAAACCATTGGACTCAG
CTCCCAAACCGTGGACTTCAAAGAGAAGCCCAGCTCCTAGCCAAGGGATCAGAA
GCCCTGGAAAAACTGTGTGCCAGTCATCCAGAGGACTTTGCTCCCTGGCGGG
GAGCAGAGTGGTAGTGACACGGACACAGACAGTGGCTACGGAGGCGAATTGGAGA
AGGGTGACTTGCAGTGAGCAACCTACTTCAAGAGCGATCACGGACGCAGGTT
ACCGTGGAGAACCGTCAGCACAATTAGCAAGAATCTGAAGAGCCCCCACCAA

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AAAGAGCCGAATGCAGCTCTCAGATGAGGAAGGCCACTCGTGGGCAGTGACCTGA
TGGGTTCCCCATTCTTGGGCCTCACCCACATCAGCCTCCCTTGCCTGCCCTCTAT
CTCATCCCACCATCGGCCACTGCCTATCTGCCTATGCTGGAGAAATGCTGGTATCCG
ACCTCTGTGCCACTGTTACCCAAGCCTCAACACCTCAGCAGCAGGCCCTCCAGC
5 TTCATGAACCAGACAAGATCCAACCTCCCTGCTCTGCCAGAAATCCCTCTCCCTG
GCACATTCGTCCCTGACTCTCAAGCCTGCTCAAGCCCTGAAGCAGATCCCTCCTTA
AACTTAGAAACAAAGATAAACCTTGAGGGCAATCNCTGCGCCTGCTTCCTTCCA
CAATTCAAGACACAAAAGGTCTGTACTCAAAACAGAGAGATCAGCCCACCCCTGCAG
ACCCACAGAGAAGATTCAAGAGTGTGAGAGTGAGTGAGTGTGCGTGCCTGCGT
10 GCTTGTATGTATGTTGTATATGTAGGACAATAAGTTCCCTCTGACACAAAGGGAGAC
ACGAGAAGGATAGCCTGACATCAGATGACAGACTGGAGGACTGTAGCACATCTCG
GGCGTTCCCTACCCAGAGAAGAGCC-3' (SEQ ID NO:8). This clone is similar to a basic
helix-loop-helix polypeptide.

Another IEG nucleic acid clone was designated L003. The following nucleic acid sequence is within the L003 clone: 5'-GCACGAGGGAGTTATTCCACGTCT
CTTAGGAAAGCCTCGTTGGTACACATGGCAATGATTGCAAGCAGATAACGTCTT
AACACCAGAGTACAGTACACACACATTGAGCTGCCCTCGTCAACAAGCAGTTGCA
GTTTGTAAATGTGAATATCTATGAAACGAGCAAAGCAACTTCCAGAGTATAGCT
TATCACAGAATAGAACACATGGGCCGCTACTGTATCATACAGAGTACAACACTATA
20 GCTTTCATCCCCGTGTGAGCATTCAAATCACTCAATGAGCACCAAGCACGGACA
AGTGAATAAAAGGCTAGTCCAACTCTCCCGCAACCCTGGCGGTAAAGGGTAAAG
AATTTGTTCAAGTAAGTTCTCCTCGTCTCTCTTCTGAAGACCTGAGCAAAC
CAACATTCTAAACCACCCCAAGATATGATACTAGAATTAAAGGCCGATGGCTCA
ACCCAGAACCTAACCTACTAGATAAAATCTCTCGAATCTGACTCACTGATGCTGT
25 TAAGTCCGACAGTACAATCACATAGTACCTCTTGATACTGTCAAAGTTGGTTAA
AAATGCCCTAAGAAAACCAAATCATTGGGAGATGTTCTAAGCAAGCTTCCAAC
ATATAAAGAACAAACCATGTTACTAAAAACATGGTGCAGGTCCACAAAACATT
TACTGCTACTACCAGGAAACCAAGCTACTCTGGTTGTGCTCCTGGTATACTGG

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TGAGCTTGGACAGCTGCTGGCACATGTCCACTGTGTCGTTATAATCAAGTGTC
AGTTTCCACTCGACAGAGATTAAAGACAATAGCTAAAAGTGAATGAAATTCA
AGTAGAAGCTACAATTGAATGCTACTTGTGAGACTTTAACATTCACATCCAAATA
TCAAAAAACTTAACCTTGACGACACATGCACACAAACACACCATTGGAAAGGGTCT
5 TGTTATGCAGTTCAAGCTGGCCTGAACTCATGATCTCCTGCCTCAGTTCTGGCA
GTAGCACTGGACCTTACTGTGGCAGAAAGTATTGCTCCAATTAGAAAGCATTACTA
TACACTTCACTCGTCATGTGCCTAGTGTGGCTCTGAAGGCATAGGAACAATGAAAT
TAAATTCTCAGCAGCTGAGGATTCTCTACACTCAACATTCTGAACATTCAATCATGG
CTTCACATTGAGGCTGAGCTAGATACAAAAATATCAAAACATCCCATAGAATTGTT
10 TATTCCCTATGTTACTGTTACCCAAGGAATGTGAAGACTAAAAAGGACTCATTG
GTTGTTAATTATGATTAAATTATGTAATATACAAACATTAAACAAAGCCATCATA
TTCCAATCTTACGAATTCTAAC TGCTAGCAGTTGAGCAGCTTTAGATATCACTAA
TAAAATATACAATTAAAATAGTCGCATTCAATCCTACTAACTTATAAAATAACTTCT
TAGGTTAGACTTCTCCTGCCCTAAGTTATAAGACAGTCTAAACCCAAA ACTCAACA
CATATTAAGCTTTAAAAACTCCATATAGTTCTAAAGTAACCTCAATGTATTCCCAA
GAACCGCCACCATCAATCAGCTCACTCCCTCACACCACTGACTTTAAGACGCTCCTG
GGTGGAGAACTGCCAGGCAGAAGCTCACCTTCTAGTGTGTGGTGTGCTGC
TCCTAGTCCAGATCTGGACCACATCAGCACAGCATCAGTGTGACTCAGCACTGAGGC
CTTGAGCGCTCTCCCCCGATGGCCTGTGTAGAGGTGTCTAATTCTGTGTATA
GATGGCCTGTATATAGAGGTGTCTAATTCTGGCTCTGTATGTATAGGTAAATGTGAT
ACTTTACCATTAAAGCACTATTTCTCCATTCAAGAATTAGTGTATAGGAAATG
AGTGGACTTGCAGACTCAGAAAAACAAAACATAACCTGTCTGAATTCAAAACAA
ACCATGGGTGTAGGGGGAACTGATGAAAGTTATGGGTTAACCTAGGTAAATTAA
CTAAGACAGTCACGAAACACATTATCAAACATTCAACACCCGGATTGAGCTTATCCACAACACCTACA
25 ACCCCACTGTGAGACCACATCACAACCCGGATTGAGCTTATCCACAACACCTACA
CCATAGTAACGCAAAGTGCACAATGTACTAAAATAATTCTATTAGTTATGCAA
ACTATGGTATAAAATTATCACCTGCCATACATATTGCCATGGCACCAACTCATAT
AATAAGCCAACGTATAATCAAAGCCTTACCAAGCACCAATCAATGTCCTGGCACCA

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CTGGACACTCACCGTCAAGCTGTCATCTAAGAGCCAGTCTGTTCTGACCTGAACAG
TTGTGCATTCCACCTTACCAACACCCAAGTCTGTGAGCCGGACAAGTGTAAATGCA
GTTTACATCTAACGGTGCAGGTTAACGCCGAGCACTGAAACTGATCACTCATTAAT
ACCTGTCTCCCTCCATACATGTACACCACATGTACACAGAACTATGTGCTCTGACTTC
5 AGAATAGCTCTCCTGTTGGCAAAACACCAACAGACATGAAGGGCCTAGTGTGAAG
CGAGCTCACAGAACATGTTGGATGGAACCTCGACTATAATGGAAACACCTGCAAAAGC
TTTGCTAACCCAGCAAACACTCAACACTTACCAAAGACAAACAGGGAAGTTAAAGTT
AGCTCGCCAAGAGAGATGGGCTGGGAGGTGGGGGTGTAACTCAAAGAAAGCTTACG
TAACAAAAACGAATGATGGACAACCTCAGAAATTCCCTAAAAACAGAACCTGAAAG
10 TGCAGGTGAGGTTTGTCTTCAGTAACAAATGCAGACAGATTCCCAACAGGAATAA
AACAGTCTGGGCTTGAAACCTGCTAGATGGAAACACGAACACTCAAATGTGGAAC
CAAGGAAAACCAAATACTTAAATGTGTAAGATAATTATAATAGTAAAAGTTGCA
AATTGCTGTGACTTGATTGCCGAAAACATCTGAAATCCACACTGGCAGTTAGAAG
ACCAGTTCCCACATTAACCTCCTCTCAGCAGGTAACCGTTGTGCGCAGAAGTATC
15 TGAAACATCGCACTACTGCTTATTATGGTGTATTGTGAGAATCTGTACATGCTAT
TACAGACAATACATATTGTAACCTGGTCATGCAAAATCAGTGTGTACAAGGGGAT
ATTGTTAACGCTTATAAAGTGGTACTTTATTATCTTGTGACGATGCCAATCTCTCCG
AAATATAGCATATCTTAAATGGTATTCTTATCTGCCAGTTAAATCATTATGTC
ACTGAAAGAAGAGGTTATACAAGGAAAGAACATGGCCTTGTGAGAATTGA
20 TTTAAATGAGAGAATTACAAAACCAAGAAATCCATGGTCATAAAGTTAACATT
TTAACCTACACATTACAGGGCAAACAGATACTGGACCCATTCCACATTCCATAA
ATCCAAACTTAGTCCCATTCAAACGTTGCCCTAACCAACTAAACCATCAGTGGT
CTTACAACCTCTGGATTATGGAAATACAGATTCTGAAGTAAAGCTACAAAAACAA
CAATGGAAGAAAGCTGAACAAACTCCCATGAATGAAAATAAAGTGGAACATCCT
25 GAAGCTCTAGACACTTCTCTCCGTCTATGGTCAACTTGTGGTTCAGTGCAGTGT
GCGGTCAAATGTAATGGCCTCATGTGGAACACACGTCTAACTAGTGTCCATTGATT
CCAAGTTAGTGGACGAAGAACATTTCTGGATACTTCAAAGATGGCTGCCAGCTCCG
GGTGGAGCTGATCTGTGACTGGAACACTCATGAGAGGGCTTCTGCCTCTG

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GAATGGTGAGCAGTGCAGCTACTGCCCTCATGGCCGAGCGCTTAACTCGTCTGCT
TTCAAACTCCTGCTTACAGAGTTCGCCTCACCTTAGTGTACACGTAGCTCGTAG
TGGCTCAACAAGCCGGTCCAACCTCTGTAGTACTGCACTTGGACAAAGGGTAGATAG
TCTCACCAACATTAAAAATGTTAGCATCTTAATATCATAATGGTCCTCAAACCATCT
TCCACATGATTAGAAATTCAAAGATATCCAGTCTGTCAAGACAGCTGTCTAGAAGT
GTGTACATACACTCAAAAGCTGCCTTCTAATGCCAGGCCGTATCAACCGTGTGC
TTAACGGGCCATCTTACCTCTCTTATAAGTCCTCCTAACTTTGTCTATTGTA
AAGATGTGGAAGAACAGAACATCCAGAAGGTCCCCTACAGTGACGGCTTATGGG
CTGCAGAATTGAATGTGACCAAGGCCACTCTTCTACATTCAAATCTGGGTCTTCCA
ACGTTTTAGAAAATCACCTATGCAGTTCTGAGCAGTGGATCTTCTTGACATCTGA
ATAAAACTGACCTACTACAGCCGGCCCTTTAGGGCATGCTCGAGTAAGGGCAGCT
ACACATTGGCAATGGAATAGTAAGACTGCTTATGAGTAAGAGCTGTGCTTGAGAG
TAAACTGGACCCGTTAGCATGCGCAGCAAATCCATGTATCCTAGATTGTTGTTCCA
GTGACAACCAAAGCTTGGAAAGAACAGTCTAGCATGGCACTAAGAGCTCCTCCCTGCAG
CAGAGGTGACCTTACAAGTCAAATCAGTTCTTGTAGAATAGATCCGCTTATCTTGA
AAGGGAGGAGGGATATACTTTGCCAGGGTAGTAAGGAAGCTGATAGCCATCTGGG
ACACGTGCATATCACTTCGCTGATAAGAGGAGGGAGCTCATCCAGAACTGCATCAA
TCATGGCGGCCGTCAAAGTCAACTGTCAGTATAGTTTAATGAGAATATCTAGGGCAGAGA
GGGTCCCCAGTTCAAAGCTCTGATTTCCTGAGAAATGAAGCAAGGATAGGGA
CTCCCTCTCCCAGCACAGGCCTCAGATCTATCTTCAAAGGTGACCCAGCAATCAGGG
TCAGTGCTTCACTGCGTTAGCCGGTGATTCATTCTGAGTCTCTCCAAGAAAAT
CTGAAGTGTATTGATAAGTCAGGGCCAAATTGTCTCCAAGATTGCAAATAATCTG
TCCCATAACAGGAAATAGCCCTCCTTGACTTCCTGATCAAATGTCAGCTGCTTTAAG
CGCTTAATTGTACAAGTGAAGAGATCTTGATGTAAGGCCTGCATCGAAGGAGGA
GGGTTGGTCCAGAGGACGGATTACTTGACAAGCTGCTGAGTGACAAGAAGGGCTT
CTGATGTGATCTGTAAAATGGTCACCAACACAAGCCACCACTGGAGGGACCAAA
GCCTGAACATGCGGGTGGAAAACCTGCG-3' (SEQ ID NO:9). This clone is similar to a
TATA-binding polypeptide (TIP120).

Another IEG nucleic acid clone was designated L048. The nucleic acid sequence of the L048 clone is as follows: 5'-TCGCCGCCCGAAGTCGCGCAGCTCCCTGGCGAACG
CGGAAGCCCGAAGAGCGCCGTCCCTGGGCCCTGCGCGCTCAGGCCCTCGCGCG
CCTCCTCGCTCGGCCGGGACGTTGCTGTGGAGGCAGTGGAGGCCGGCGGTCGAGCA
5 CCTGGAGCGACGGTAGCCCGCGGCCTGCGGTTCTCTCCTCCCCGCCGCCCTCCA
CCCGAGCTCGGGGGCTCGGCCCTCGGTGCTGCACGAACAAAGGAGGCC
CCCGCGGCCGCCGCAGCTCCATCTGCGGTCCGATCCACCCGGGCCGCCGGGCC
GCTAGCCAGCCCTCCCAGGGCTCAGCCGGCCCACCGCCGCCGTCGCGCGCCA
GCTCGCTAGTGCATCCGGCCCCCAGGCACAAAAATATGGCTCAGGAGACTAACCC
10 AGACCCCAGGGCCCATGCTGTGTAGTACTGGATGTGGCTTTATGGGAATCCTAGGA
CAAATGGAATGTGTTCTGTTGCTACAAAGAACATCTCAGAGACAGCAGAACATGTG
□ GCAGAACATGAGCCAATGGGGACAGCTAGTGGTCCAACAGTCCTACCTCAGACTCTG
CGTCTGTACAAAGAGCAGATGCTACTTAAACAACGTGAAGGTGCTGCTGGCAGCA
15 CATCTGAAAAATCAAGAAATGTGCCCTGGCTGCCCTGTAACACTAACAAATGA
CAGAAATGAGCATTCAAGAGAGGACAAAATAACCTCCCCGAAAACAGAGGTGTCA
GAGCCAGTTGTCACTCAGCCCAGTCCATCAGTTCTCAGCCAGTTCTCTCAAAGTG
AAGAAAAAGCTCCTGAGTTGCCAAACCAAAGAACAGATGTTTATGTGTAGA
AAGAAAGTTGGCCTTACAGGGTTGACTGCCATGTGGAAATTGTTTGTGGACTT
20 CACCGTTACTCTGACAAGCACAACTGTCCTATGATTACAAAGCAGAACAGTCAGCA
AAAATCAGAAAAGAAAATCCAGTTGTTGGCTGAAAAAAATCCAGAGAACATATAAAA
TTACTACATGTGAAGAGACTGAAACTTGTGTTTATTTAATATATCGTAGGAAAAC
ATTAAGAGCAGATGCATGCCATTTCCTTGATGTTCTCCAGAGTTGCTTATA
CTTGTCTGTCATATAATTGATATTAGGATGTTGGGTGTTACAGGCAGAATT
GGATAGATACAGCCAACAAATGTATATGCCCTCCCTCAGTAAAATGGACAAAAA
25 ATATGCACAGCAAATTGAAATACACATATACTAGGAACAAAATTAGTCCATGTGC
CAAACGTGAATGAAATCTCTGCATGTTGCAGCATATCTGCCCTTGGGAATGTAATC
AAGGTATAATCTTGGCTAGTGTATGTGCCGTACTTAAAAAAATGGTACACCAG
AAAAGGACTGGCAGTCTACTACCATAGTCACCTAACCTTAATTGACATGCTT

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TGGAAGCAGGAAGAAAGCTACAAAACCAGTATTGGTGCATGTGTGAGCCTGGTT
AAATTGGTCTTCTAAAAGCTGTCAATTAGGACATTCTCGCAAAGGTAACATCACAAAC
TGGTTCTGAGTAAAACCATCAAGTCACAGCAGGGTGCCTGAGATAATCTTGAGC
TTATTGTGCTGGCCTGCACCAGAAGATATCTGCATTCTCATTACTAAAATTGTAGCAC
AGAACTGCACTAGGATTGTTACAAGAAGAAATTAAAACCTACGTTGGTTCA
CATATAGCAGCTCTGTTAAATAACATGCATCTGAATTAAAGTTGCAAAGGTATCTG
AGCAGTTAGTTTCATGTGCATCTTGTGAATGTTGGTTCAAGAAAGAATGTT
TAAAGCTTTAAAGACTTCAGTTCTTAATGTAACGTACCCCTGCATGGAAAATC
ATAACCAACATGGCTGCAGTAGACTCTTAGTGGTATCCAGCACCAC TG CAGAGG
GCTGCTTATCATATTGTATTGGGTGTAGGACTCTAGTGTCTGGGTGTATTGCAT
GGGCTGCATTATCTACAGCATTGTACAATAACAACTAGAAAAGGCAGTATACTTCAC
TGATGCTTGTCTGTAATATCACTCTGTGTATAATGGAAGGTTTTGTGATGTAT
GAAACTGTGTTTTATATATAATGAGTATAGTTAGATTAGTGTGTTGTAATGCC
TGTGTTCATCTGAAATAGTTAAGTATGTACACAAGGCACTACTCTGATTATTGCA
GTGTTCAGTCCTAGTTCTTATTAAAACATTCAAGTTGCTCAATTATGTACT
TTAGTTCTAAGTTAGATTGCAGATGTGTACAGATAGTTCATATTATGTATTGCACA
TAATCATGCTATTCA GATTGATGCTATTGTATTATGTAATAATAAAAGCAGTG
TACAGAGGGAAAAAAACTCGTGC-3' (SEQ ID NO:10). In addition, the L048 clone
contains an open reading frame (ORF) from basepair 414 through basepair 1055. This ORF
encodes a polypeptide of 214 amino acid residues. The amino acid sequence of the L048
polypeptide is as follows: MAQETNQTPGPMLCSTGCGFYGNPRTNGMCSV CYKEHLQRQ
QNSGRMSPMG TASGSNSPTSDSASVQRADATLN NCEGAAGSTSEKSRNVPVAALPVTQ
QMTEMSISREDKITSPKTEVSEPVVTQPSPSVSQPSSSQSEEKAPELPKPKNRCMCRKK
VGLTGFDCRCGNLFCGLHRY SDKHNCPYDYKAEAAAKIRKENPVVVAEKIQRI (SEQ ID
NO:11). In addition, the L048 polypeptide was found to be cysteine rich, having a motif with
distant homology to that of polypeptides with Zn⁺⁺-fingers.

Northern blot analysis using a sequence from the L048 clone revealed the presence of a 2.5 kb mRNA transcript. In addition, this analysis revealed that the expression of the L048 mRNA was strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated L064. The following nucleic acid sequence is within the L064 clone: 5'-ATTCCAAAAATGCATAGATTACAAAGAACACC
5 AGACAAGCTCAAACCAAGGATATTCTACAAATAACCAGTACCTCAAAATGCCAT
AGCTACCAGGTACAGACAGGCGAGANACTGTTCCACACTGAGGAAACTAACAAAGTA
TCCATGAAGTCCATAATTGTGGTCAAATCCAGGACCTGCAAAGGGGATTGGGAT
AATTTCAAAATTGACTAAGGTCTGCAGAGTAGAGAGACGAGGTCAATGCCAATGT
10 CCTGATTGACAGTAAGTATTAAATATGCAGGAGAACAAACCTAACCAAGAGGCTG
CCAACACACTCCTGGCTGTGGCACAACTAGATTAAAACCAGCAATTGTTGGTTC
TTGTTCTCAAATATCAGTTACCTGCAAGCACTCCATCGTCAAAGGATTGAGAGCATG
AGGTGATGTGTTGATGGTAAAATGAGAACTGACTGAGCACAGGAAAGAGTGGCAT
GATGGGCAGGGAAAGGGGAGACAAAGGTACAAGAGCATGCAACACTCAGTGAAC
15 TACAGGACACTCCAAAAGGCACTCTGCTGTCTAGCTGGATCTGGAGGAGGATCAG
NTATTAAATAAGGCCCTGGAAGGGNCAAAGCTAGCCTCCAGCTGCTGGCTTCCAT
CTGCT-3' (SEQ ID NO:12).

Another IEG nucleic acid clone was designated L067. The following nucleic acid sequence is within the L067 clone: 5'-GCCACCACCATTGTTAATGGAGGGAGGCTCTCC
20 CTTGTTATTCTCAGAAGACTGAATGTCTGTACCAAAAGGCTCATGGCTTCTGGG
CCTTCATTAAGGTTAGTTTATGTAGTACTAAAATCTAGGCTTACT
AAAGTGGCTTGAGTTATTGGTATCGGTGGATTTATGTTACTTGGAGTCCAGAA
CAGGGAGAGCTCACCAACCTCTCCTTCCCTGGACCAAACACCCTCTGTCCT
25 GTGAACTCACCTTCTGTGGTCACTCCCATTACCAACTGGTGAGCGAGCC
AAATGGATGAGAGACACAAAGACCGTAGTTCTGAGAGACATTATTTTCAACTT
TGTGTTAAGAGATTATGTGTTGATTGTTGGTTAAAGGGATTCTA
GCTAACTGGATTTGTTACCTCAGCTCTGGAGAGGATTTGCTGAATGACTATT
AATTACCTGAGCATTGTTGCTCTGAGGTCAATGGCATGCTAGCCTATGTCTGTTACAGT

CTCAGGCTGCCCTGTTCCCTCGTCTGTGCTATTGTGCTACACGCTCAAGGGGCCT
TGACTCTGCTTACACACATTAGGGGCAGTGTGAGTAAATGTGCAGTGTCCACACTG
AGGACATGAATGTCTGCACTGTCACTTTGCTCTGGGTGTGAAGTCCCTGGTCCCCTG
CTCCTGTAGCTTCTTTGATCGACTACTGGAACCTCAACCCTGTGTACAAGAGCAGC
5 ACTGCCTCTGGTGGTGGTGGTGCAGCCAGGATTAGATGCCAGTCCTCGGGTCCC
TGGCCTTGTGGAAAGGTGTGCTTCCCTGAGGTCTGAGAATGGAAGGCTTGCCTCA
CTCTAGCTAGGAGGCGCAATGGGAAAGTATGAGTTCAGGGCGTCAGGGCAGTGGCT
CCTGAAGAGGCCAGCTGTGGACAGAGGGAGTGAGGCTTATTAAAGTGACAGGAAG
AACATGGCGTTTGGTATATTGGGAGCAATGCCAAGATTCCCTCCTGCCCTACATA
10 GGTACACAGACACCTTCCCAACCATCCCCCTCCACTTCCATAAAATGAAGACAGCCC
TGATGACCCTCACCCCTTGCATAGGTCACTGGATCCCCTGTCCTCCTCGGTGCT
TACACACTTACAGACCCTTAGGCGAGCCCTGCATAGAGCGTTATCTCAGTGCTC
CATTCCAGTCCTGACTCCCTGTGGCATTGAGACTTGGATTAAAGAACTCACATTGC
TAGGGAGAGGGGCTTGCTGGAAAGGTGACTCCTCTGTAACCTAGCCTTGTGCT
CCTCCATGACAGAAATGCTGGTGGAGTTACATTGCCAATGCCAGCTTGTGAA
TATCTTCATATACACTTCTATTCATGTTACTGTAGTTCTGTTGAAATAAAACTC
TGAATGT-3' (SEQ ID NO:13). This clone is similar to a glucose transporter type III
polypeptide.

Another IEG nucleic acid clone was designated L076. The following two nucleic acid sequences are within the L076 clone: 5'-CATATAATGTACTTATTGTTAAACAGAACG
20 AAAGAAGAGGCAGAAAACATTGCATGTAAGTCCTAGCTTAAATGTAGTTTAG
TGGTGGCATCTCTAACACGTCGTCAGGGACTGTTCCCTTGCCTCCTGTACTGTG
AGCACTGACACTTGAGAAAAGCACATCTGGCGGACATATGTCTCCAGAACCTGGAAAG
AACTGGAGAGCAAACATTCTTAATTCCCTCAAGTAATCTTAGTAAACAAAA
25 GATGATCTTGGCATAGATTCATACTTAAAGGCATTGATATGCATTATATCAGGTA
AGCAACTATACAGATCTGCTGAGAGCTTCAAAAGAATCTGTTATCAGCTGAAAGGA
AATAGGGGAAGCCTGAGTATTCAAGGGTCAACTTAAGATTGCAAGTTCAGTGTGGG
GTCAACATACTAGATGTGGGAAGAACATCCAGGCAAGGTCTAGCCTGTATTCAACC

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TGGTTCTGATTCTGGAAGAAGCATCCATGCGCTAGGAAATGCTTACAGCCGAG
GTAAATGCAAAATGAGTAAAGTCACTTTCACTAACTTGCCAATAGGRAACAT
GCCTTCTGATAAGTAGATACCATACTCTTATTCTGAATACTTATATTGAGAGAA
GGTTGTAGTTGGTAAAAGCAACTGGGAACATAACTCCTACTGATTTCCCTAGC
5 AGCACCAGAATTATATTCTGCAAATGCTATTCTCCCTACATAGGAAATATCCTCA
GACAAAATTGCCTTCCATTCAAGTCTCTTAAGAGYTTAATTGAATGGACTTTCAA
AGTTACAAGCAAAGTCAAGTGTGGTAGGAGCTAACAGAGGCTGACACAAGTAGAT
GACTTGAATCCAGAAGTTCAAGACTAGCCTGGACAACATAGAGAGACCCAGTCTCA
AAATT-3' (SEQ ID NO:14) and 5'-GGCGGGATCTCTCGGCTGGTAAGAAGGGG
10 CAGTGGTACCANGCGGGACTTATTCAAGTGTGCCAAGGATATGCCAAGGCCTCTGA
TGAGGTGACGAGGTTGGCCAAGGAGGTTGCCAAGCAGTGCACAGATAANGCGGNTT
AGAACCAATCTCTTACAGGTCTGTGAGCGAACCCAACTATAAGCACCCAGCTCAA
ATCCTGTCCACAGTGAAGGCCACCATGCTGGCCGGACCAACATCAGTGACGAGGA
GTCTGAGCAGGCCACAGAGATGCTGGTCATAATGCCAGAACCTCATGCAGTCTGT
15 GNAAGAGACTGTGCGAGAGGCCGAAGCTGCTCAATCAAGATTGANCAGACGCCG
GATTACTCTGCGCTGGTCAGAAAGACTCCCTGGTACCAAGTGGCACCTGGTCAGA
CCTGGCTGGTACACAGACCTCTGCTAATGANGANGTGACCATCTTGAGCTTCAGAAG
CCATTCAAGAGTTGCCAAGGGTGGNAAATCAATCCCTGGTTCACACACCAAGAAA
GGGAATGGGCCTCCTCACATTAGAATAAACATTATACTCTGTCATGGGACACT
20 TTGAAAGTGTCTCCTACAAAACCCCTGGTACCTTCAGGNTTACTCCNGGTNGCA
ANNTCCCTCCCCAAGGGAAATTTTACCAATAAAAGGCTCAAGGAATTAANGGCG
NTTGAAAACCAACNTNATCCAANGGGAAANGCCCCNTGGCCTCTGGCCCCCTGG
GGGNACAATTTCNCCNCTGGTAAATGGGTTCAACCTGGGCTGG
NCCTTTCCNCCCCCTTAAGGGCTCCTCGAAGGAACCTNAGAAAACCTN
25 AAGGGCAAAGNTCCANTTACNAATAACTGGG-3' (SEQ ID NO:15). This clone is
similar to vinculin.

Another IEG nucleic acid clone was designated L082. The following two nucleic acid sequences are within the L082 clone: 5'-TTTTTTTTTTTCCCTCCCTAAAAGAT

5' 3' 2' 1' 0' -5' 6' 7' 8' 9' 10' 11' 12' 13' 14' 15'

AAACTAATAAACTCTCAATGGTCTTCAGTATAGTTCTATGTAGTTAACATAGC
TTATAAATTGAGTTAACAAATAAAACTCAAGAAGATAATTTATAAACCCTGTTTCC
AATCTGTCATTACTAAATTATTTGGTTGTTCCCTTTCTTCTCACCC
CCTCCCTCTCCATGAAGATTCAAGGTGCTAACATATCATTTCCTGCTGGAATT
5 TTAGCATTGATATGAACCATGGACAAGTATATTCTGCTGCCACAAAGACTGTAAAGT
GCTTCATTCAACAGCTGAGGCAAGCCAAGTGATCATTAAATAAAGCTTCTGCTTC
CTTCAGTGGTGGTAGTAAAATGGTAGGTAAAAGTTAGGCTGCAAGTTCAATAAA
TGAGATTACCTATCATTCCACCCCTGTGTATTCACTACCTATCCTGGTTCAAGCAG
TTTGAGTCAACTAGGCATTAAAGGCATTGTGTTATTACTTATGGTCCAACTTA
10 CATACTTGTCAAGGATGAAGTCTGATAGGTTAAGGACAGTAGAAATTCTGTGCAAC
AAGCAGCAAC-3' (SEQ ID NO:16) and 5'-TTTTTTTTTTGGTTACAAAGT
ATTATTTATAAAACTTGTATTAAAATAGAGCTTATCTGTCTACTCACAAATCCTA
ATTAAAACATAACACATTATCCTAGCTAATCTGATGTTAACCTTACAATCAACAC
TCATTTTGTAATTTATTAAGAACCTGTACTAAATGAAGTTTAATCAGAAAACAT
15 TCCCTTTATCTTAAAAGTGCTTCTTAAATGAAGGCACCAACAAGAAACTACTTCAG
ATGGTACAGAACATTCTTATTCTGAAGACTCTGTGGTTGACCACTTCTCATTAGTT
ACCTGCAGCAAGACACCTCCTGCCAAAGGAAAAAAAGTATCTGAAGAAGTT
ATCATGTTGTCCAAAGAACCTAAGTAACCTCAGTGGTGGTTAGGATTAAAGCAG
ACTCACTGATGTGTACGCCCTGAATATCACATTCTGGAAAGGCAGTAAAGCCTA
20 GAAATCAGAAGGCGGGCGGTTAAAGAAATTCAATAGCCAACCTACAACANTTT
AGGGCAAAGATAATGGGAAAAANTNC-3' (SEQ ID NO:17). This clone is potentially
similar to a nRNP polypeptide A2/B1.

Another IEG nucleic acid clone was designated L094. The following two nucleic acid sequences are within the L094 clone: 5'-ACGATATMTAYWGARRTWYAWCTSTTHAC
25 TGAATMWHATGCACAAATATTAACACTAGTRRTTATTAAACAGATATSATTAGAAC
AGACTTAAWKAATAACAAATCCTTAGGTACGRTTAATATCATGTTCADGATGTTG
AAGAGTTAAAAAGAATCACTGATTAAGKKAAGCATCCBCACTTTCTTGAGAABC
CAAACCTTTAGGNAAADACCCATTCCAAATTGTCCCCHATTCAAGRCKKCAG

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

AAAGTCTAACATSAAGAGTCCTCAACGGGNGTAACTCAVAWCTCCTATCAAGT
GCAGTAACCTAGCTCTCCGGDGGCCATGGCGT-3' (SEQ ID NO:18) and 5'-AAACT
AAACAGTGTGTTGTTAATTCTTCTGCATTGGACTATTGCAGGCATTAGAGCATCCAG
AGCTACGAAGGGCTGGCTGCAGCAGCACCGCCTTGTAAGCCAGCAGACCAGCCT
5 TAACTGTGGGCTTGACTCCTGTGAGCTGGCCTCAGTGTGACTCAGAAATGTTGATT
AGCAGATGAGAGAGCGAGGACACACCACGAGGGCTGCCTCTTCCTCCAGCGCT
GTGCAGGACAGTTCTCACCCTAGCCTTTAAATGCACCAGAAGTACAGACAG
TTGCACTACACAAACCCTTGAACACTTGTAGAAATCAGTCCACCGTAGATTAGACA
GAATCACCTCCAATCCTTGACTIONTCTTCTTCATTGAACAAATTGTATAATAATT
10 GATTATTGTCAAATTTGTCTGTGGTAGTATCGCTTAATTATCTTAGTACATCAA
CGTTTGATTTAAAAAGAATTAAAACAACAAAAAGTCACTTAGAACCCATGAA
CTTTTTTTNGATNGGAAATTCTTCTTNGAAAATTATCATTGGGTTCTCC
GGAAANCTGTAAGATTGGNTATAAGGTACCTGGGANGTCANAACNGGTGGNTA
TACCCCTTTTAAGGGAAATTATGATTNGAGTTTGGGCCACTNCGGGANTGG
CAGGGAAACCANNNGGGNGGGTTAAATTNTGTGAGGGTTTGGCCTNAA
TTTTTGATAATTTCACCTNGNAACCTTNAANNCTNGGAAAAAAAAACNT
-3' (SEQ ID NO:19).

Northern blot analysis using a sequence from the L094 clone revealed that the expression of the L094 mRNA was upregulated in response to the multiple MECS treatment. Specifically, L094 mRNA expression was induced 7.3 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I). In addition, developmental studies revealed that the transcriptional expression level of L094 was upregulated between day E15 and E18, and downregulated at day 0. The expression then increases again during post natal development.

25 Another IEG nucleic acid clone was designated L097. The 5'-end of the clone obtained from the first library screen was used to design an antisense primer. Using PCR, L097 DNA was amplified and inserted into the pCR2.1 vector. The L097 clone is about 4.4 kb in length. Sequence analysis of the first 4060 bases from the 3'-end revealed the presence of a coding

region of at least 2351 bp. In addition, RT-PCR analysis revealed that the L097 clone was missing an adenine at position 1166 from the 5'-end. The lack of this base results in a frame shift in the coding sequence. Further, the sequence at position 1358 was ambiguous. However, any base substitution at this particular position will not alter the encoded amino acid residue.

5 Specifically, a serine residue will be encoded by the codon containing nucleic acid position 1358 regardless of the base at position 1358. The following nucleic acid sequence is within the L097 clone: 5'-TGCAGCCGCCCTGGAACTGCATGTCAGGAAGCATTCCCTTGTA
TGTCTGTGCTATATGTCATAAGAAATTGTCAGCTCAATCAGGCTGCGCTCCCATATC
CGAGAGGTGCATGGGCGGCCAGGAGACCTGGTTTACTAGCTCCATCAACCAG
10 AGTTTCTGCCTCCTGGAGCCTGGTGGGGATATCCAGCAGGAAGCCTGGAAACCAG
CTATCACTGACAGCTGAGGAATTGTCAGCTCAATCAGGCTGCGCTCCCATATC
GTTTGTCCCTGGGAAGCTCAGCCTGAGGTGGGGCTGAGGGAGTTGGAGGCCCTGG
AGAACATGTGCCCTGGAAACCAACTGTGGTCAATTCCGACCTCAACTCTTGGAGT
GTCCCCCTGCAAACACTGGAAACCAACTGTGGTCAATTCCGACCTCAACTCTTGGAGT
GGTTTCAGATGATTTTACTGAAAACCTGATACTCTCTGCTGAGCCTCATGCTGCT
GCTGAGCTAACCTCAGACACACAGCATCGAGGCTCAGCCCAGACTCAGGGTGAAGA
AGTCACACTGCTGGCCAAGGCCAAAGTACTGGACCAGACTCAGAGAGTCCTC
CAAGTGGAGGGCAGAATGTGGTGCTCTGCCAGCCAGTGAATCTGACTCTAACAGG
TGTCTCAGGGCAAACCCAGCAGAGACCTCAGACCTCCTACAGTGGCTGATGGA
GGAGACCTCGGTGTGCCAGCCTGACTCTGCACGTCGCTCTGAGCACCCACACCCT
GGCAGCACAGCATTGTAAGGTCTAGACAGTCTCCAGAAGAAGCAGATGAACAC
CAGTCTTGCAGCGGATCCGGAAAGGTTATGGAGACCTGGAGTGTGAATACTGTGG
CAAACTTTTGGTACCAAGTGCATTTGACATGTCAGTCCGACCCACACCCGGGA
ACATCTGTATTATTGCTCCCAGTGTCACTACTCTCCATCACCAAAACTGCCTAAA
25 CGCCATGTAATTGAGAACACAGTAACATCTGCTGAAGTGTCCCAGTACGGCTGT
GAECTACTCGACTCCAGATAAAATATAAGCTACAGGCCACCTAAAGTTCACACAGAG
CTGGACAAAAGGAGTTATTCTGCTGTATGTGAAAAATCTTTCAAGAAGACCGA
TTGATAAAAGTCACATATCAAGACTAATCATCCAGAGGTCTCCATGAATACCATTCT

GAGGTTCTGGGAGAAGAGTCCAGCTCAAAGGGCTAATTGGAAAGCGAGCCATGAA
GTGTCCGTATTGCGATTCTATTCTATGAAAGAACGGCTCAGACCTTCAGCGGCACAT
CTCNGCTCACGAGGGTGTGAAGCCCTCAAATGTTCTTGAGTATGCAACTCG
TAGCAAGAGCAACCTCAAAGCTCATATGAATCGTCACAGCACTGAGAAGACTCACC
5 TCTGTGACATGTGTGGCAAGAAATTCAAATCCAAGGGACATTAAAGAGTCATAAG
CTCCTTCACACATCTGATGGGAAGCAATTCAAGTGCACGGTGTGACTACACAGCT
GCCAGAAACCACAGCTGCTGCGACACATGGAGCAGGATGCCTCCTCAAGCCTTTC
CGCTGCGCTCACTGTCATTATTCAATGTAACATCTCTGGATCTCTGAAACGGCACTACA
ACAGGAAGCACCCAACGAGGAGTATGCCAACGTGGCAGCAGGGAGCTGCAGCT
10 GAAGCCCTCATCCAACAAAGGTGGCTGAAGTGTCTGTTGCAGCTTGTATGGA
ACCAAATGGAGTTCAACAGACACTGAAGAACAAAGCATGGCTGAAGCCAGCGAC
AGAGACTCCCGAGGAGCCCTCACCCAGTATCTACATCACCAGGCTGAAGATGT
TCAGGGGACACAAGCAGCTGTAGCTGCACTTCAGGACCTGCGATATACTCCGAGA
GTGGTGATCGACTTGACCCACAGCTGTGAATATCCTGCAGCAGATCATTGAACTGG
GTTCAGAGACTCACGATGCTGCCGTGGCCTCCGTGGTGCATGGCCTGGGA
CAGTGAUTGTTGAAAGCAGGTACCGATGAGGAACCAATTCCAACCATACTG
ATGATCCAGGAGACTCTGCAGCAGGCCTCTGTGGAGTTGGCCAGCAGCACCATCTG
GTGGTGTCTGATGACGTGGAGGGCATTGAGACAGTGACAGTGTACACACAGGG
TGGGGAGGCCTCAGAGTTCATCGTGTACGTGCAAGAGGCTGTCCAGCCATGGAGG
AGCAGGTCGGGAGCAGCCAGAACACTAGAGAACCTGCCTCCTTGGC
AGCCAGCCTTGTGGCCTGAAGACCTCCTAACCCACCAGGTCCATCCCTGGCTCT
CTTGCCCAGTGGCCCCAGATAAATTCTCCATAACTGTCTGTGTGGTCAAAGCCA
GGAGAGTATCATGAAGAGAGAGAGAGAGAGACTAGTCTCCGAGTTTTTTT-3'
(SEQ ID NO:20). In addition, the following amino acid sequence is within the L097
25 polypeptide: QPPELHVRKHPFVYVCAICLKKFVSSIRLSHIREVHGAAQETLV
FTSSINQSFCLLEPGGDIQQEALGNQLSLTAEFVCPEIDVRKGEVCPGEAQPEVGLRELE
APGEACAPAVPLANPQSVSLSCKLETVVNSDLNSLGVVSDFLKTDTSSAEPHAA
AELTSDTQHRGSAQTQGEEVTLLAKAKSTGPDSESPPSGGQNVGALPASESDSNRCLR

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

ANPAETS DLLPTVADGGDLGVCQPDSC TSSEHHPGSTAFMKVLDLSLQKKQMNTSLCER
IRKVYGDLECEYCGKLFWYQVHFDMHVRHTREHYCSQCHYSSITKNCLKRHVIQK
HSNILLKCPTDGYSTPDKYKLQAHLKVHTELDKRSYSCPVC EKSFSEDRLIKSHIKTN
HPEVSMNTISEVLGRRVQLKGLIGKRAMKCPYCFYFMKNNGSDLQRHISAHEGVKPFKC
5 SLCEYATRSKSNLKAHMNRHSTEKTHLCMCGKKFKSKGTLKSHKLLHTSDGKQFKCT
VCDYTAAQKPQLLRHMEQDASFKPFRC AHC HYSCNISGSLKRHYNRKHPNEEYANVGS
GELAAEALIQQGGLKCPVCSFVYGTKWEFNRHLKNKHGLKPATE TPEEPSTQYLYITEA
EDVQGTQAAVAALQDLRYTSESGDR LDPTAVNILQQI ELGSETHAAA VASVVAMAPG
10 TVTVVKQVTDEEPNSNHTVMIQETLQQASVELAEQHHLVVSSDDVEGIETVTVYTQGGE
ASEFIVYVQEAVQPMEEQVGEQPAT EL (SEQ ID NO:21). Using tblast2x algorithms, nine
Zn⁺⁺-fingers were identified by homology to motifs of Zn⁺⁺-finger containing polypeptides
(accession # PIR2:A32368, S03677, A29634, S06571, and A60392). The presence of the
multiple Zn⁺⁺-finger domains suggests that the L097 clone is a transcription factor, however, the
size of the encoded polypeptide is in excess of 700 amino acids.

15 Northern blot analysis using a sequence from the L097 clone indicated that the L097
mRNA transcript is rather rare. In addition, this analysis revealed that the expression of the
L097 mRNA was very weakly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated L099. The following four nucleic acid
sequences are within the L099 clone: 5'-TGGATCTACTTGTAA TGGTTCATGGAAGC
20 AATCAGCAATATGTGATATGAACTGCTGCATTACTTATTATACTCGTGGAACTGAGA
TATT TARMSRSMGCTTWWYTTTTTTTTAGTGTAAA ACTTAAGCGTTCCAC
TATTGGAAGAAAAGCATATATGGGTATTTGTATTGTA ACTTGTTAAAAGGACAGT
CTTTTTAA YCTTCCC ACTTAA ATGCTTAAAATGTAATACAATTGAAGCTTGT
TTAAAAATAGAATTAAATGTCTTAWATAGKGCTACKGTTGGAAATTAGAAAGTGAT
25 CAAATACAAA ACATT TAAAATTAAGCCCAGAAAACAAAATAGTGTAAAGTTAG
TTAGTATAAAAGAAATTATAAGATT TTTCTTCAATATAAGATA CCTCACTGAAA
ATAAAGAAAGCACAGCACATTAAAGTAATTCTCATGAGAACACCCCCATTAGAATAA
TTGCTAAATCTAGGACACCTT GAGTTGTGAGTTGTGATACATGTAGTCACCATT

GCTTTCTGCTGGAAGGACTTCCGTAGTAATTAAAGGCAGTGTAATAGTTCAATT
CCCCACAGTTCAACCTGGGAAGGCAGTATGTGAATGGTCCCTTGCAACTACGG
AAACACATTAGCTACATTGAGCATAACTCGATTGATAATTGCCAGTGCATATA
TTTATGATTAATGCTGGTTGGTTGCATTACACGACACACAAA
5 CCTCACATGAAATAAATTTATATGGTTACTAAAAAAATGACTCATCTATCTGG
TTACTTAGTTACAAATTGGATTATATTATTGAAACATGACATACTGTGCTCTTA
GCTTATACCTCAATCGTATTTGTGCTGTTGCCATTTCATGCCTGTATATAACTTG
TATAGATTGGATGATATTCCAATAAACACTTTAATKCCA
10 WRAAAAAAAAAAAA
AAAAA-3' (SEQ ID NO:22); 5'-TAATGTTATGATACAAAGCTACTCACTCTG
GAGCCTCTCATTACAGAATCTCTGACTTTATACACCCAGCCTGTTACTTTGT
TCAGGTTGCAGAATGAGTTCCCTCTGGTTCCCTAGAGGAGTTCCCTGATGAAAT
GCTAGTAGCACCTCCCCGACATACAGCGGGTGGTGGGCACACTTGCTGTGCTCT
GATGGTACACACAAGAACAGTTGAATTGTCTTCTGTTAAGAGTGACCATAGC
TAGATATGTGTGTGACTTCAGAAA
15 ATTAAATGCTTCCGAACCTTCCGTAAAT
AGAGGTGTGAAGTACTCATTGATGCTGTGAGGAAAGTGGATTCCACGGACGCACA
CCGCTTCCTATGTA
20 ACTCACAATGCTCTGTACAGTTTATATGTTAGTCTTACAAAGG
TCTTATGAAATTATATAATGGATTTCCTTAAATTATAAAACTAAATATCTT
AAAGATTGTTGGACTTTGTATGTTAAATGTTATCTTAAACTGCACAAATGGA
CCATGATGACTCTTGATCTTAAATCAGGAATTACAGTCAGCTAAGAAAAATGTG
GATAGGTTAATAATCCACAGTGGAGTATCTGCTAGGAGCAGGAATTGTTAGATGAC
ATGAATTCCGTGATTGAGGAAGGGCAGCCTCTGCAC
25 TTTCTTGTGTTGGCTTGG
GCACATGAAGTCTGACATTTCACCATCGAATTTCACATTACTAGATGGTTGGCTTGG
GATTACCTAGGGAAATTCTTAGCAACTTGACTTGTGTTGGCTTGGCTTGG
CTCCAGCTTGCAGAGACCCCTTGCCTCTGTCTCCAAAGTGGTGGCTGGCAGGATG
AGCCCCACCACCGCTGGCCCTGTGCAGTTCTTGGATGTCCTGAAAGCAGCTGT
GGCATTATCTTCTGTTCATGTGTCGGAGCTGTCTCATGGTACTACATGCAGTGACC
TGAGATCTGCGTTAAGGAATAACTTAGGAGAAA
30 ACAGGCTGTCACTGTCCTCCCCGCT
GTGAGACACCAGAGTTACACACCTGTTAGTCATACTTGTTATGATACTGAT

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GTCTAAGGCAATTTCTACTTCCAAAAGGGAGTTGTTCTAAATATATTGTGAC
CTAAATGTGGTTTATTCTGCTATGTTCTATAATTATGTATTGACTTTGTAACCTCC
TTGGGAGAAACATGTTAAGTGGCACAGGGACCATATATGTCATTTATTAGCTCTG
GAGAAGGAAACCACAGGCAGTTGAAAATAGCATTAGCTTAGATGTCAGTCATTGT
5 GCTTGGCTGTGTGGGAGGCAGACTCAAGGACTTGACCCATTATTTCTGACAGAA
GTGTTCTGCTTATGTGCTGCTTAGTAAGTGTGATTTCAGTCTGATGAAACTTGC
CTCGTACATTGTTGAGCGTAGTCTCACTTCCAGAAGATGAAATGATGTGCCATC
ATTTCAGTCTAAACTCCTTAAAGTAATTAACTCAGCTGAAATATCATATCTC
CTACTGTTGAAAGTAACTTAACATTACATTGACCATATAGCTGAAAACCAACTTG
10 AAATTCTGTACTCCTCCACAAGTGACCTCCGCTAAAATACCCATAGGAAGCTTACTT
TGTGCATGCNTGCTTGTGCCGGTGCCTCCTAANGTTGCTTGGG-3' (SEQ ID
NO:23); 5'-TTTTTTTTTTTTTTAGTGTAAAATCTTAAGCGTTCCACTA
TTGGAAGAAAAGCATATATGGGTATTTGTATTGTAACCTGTTAAAAGGACAGTCT
TTTTAATCTCCCACCTAAATGCTTAAAATATGTAATACAATTGAAGCTTGT
AAAAATAGAATTAAATGTCTTATAGTGTACTGTTGGATTAGAAAGTGTCA
AATACAAAACATTAAATTAGCCCAGAAAACAAATAGTGTAAAGTTAGT
AGTATAAAAGAAATTATGAGATTCTTCAATATAAGATAACCTCACTGAAAAT
AAAGAAAGCACAGCACATTAAAGTAATTCTCATGAGAACACCCCCATTAGAATAATT
GCTAAATCTAGGACACCTTGAGTTGTGAAGTTGTGATACATGTAGTCACCATT
GCTTTCTGCTGGAAGGACTTCCGTAGTAATTAAAGNAGTGTAAATAAGTTCAAT
TANCCCACAAGTTCTAANCTGGAAAGNAANTATGGTAATGGNCCCTCTGCAAC
TACGGGAACACA-3' (SEQ ID NO:24); and 5'-TTTTTTTTTTTTTTGGCATTAA
AAAGTGTATTGGAAATATCATCCAATCTATACAAGTTATACAAAGGCATGAAAAT
GGCAAACAGCACAAAATACGATTGAGGTATAAGCTAAGAGCACAGTATGTCATGTT
25 TCAATAAAATATAATCCAAAATTGTAAACTAAGTAACCAGATAGATGAGTCATT
TTAGTAAAACCATATAAAATATTATTCATGTGAGGTAGAGGACAGTTGTGT
CGTGTAAATGCAACCAACCACAGCAATTAAATCATAAAACTATATGCACTGGCAAAA
TTATCAATCGAGTTATGCTCAATGTAGCTAATGTGTTCCGTAGTTGCAGAAGGGAC

CATTCACATACTGCCTCCCAGGTTAGAAACTGTGGGTAATTGAACATTACACTG
CCTTAAAATTACTACGGGAAGTCCTCCAGCAGAAAAGCTAATGGTACTACATGTA
TCACAAACTCACAACTCAAAAGGTGTCCTAGATTAGCAATTATTCTAATGGGTGT
TCTCATGAGAATTACTTAATGTGCTGTGCTTATTCAAGTGAGGTATCTTAT
5 ATTGAAGAAAAATCCATAA-3' (SEQ ID NO:25). This clone is similar to sno I.

Another IEG nucleic acid clone was designated L100. The L100 clone is 2924 bp in length and has a nucleic acid sequence as follows: 5'-TGCGGCCGCCGGGGCGGG
GCTGAGCCAGTCTCTCCGCCGCCGGACGCGCAGACCTGGCAGGCTGCACCG
ACGGCCGCCCTGGCCGAGCGCACTGCAGGTCGCTGCGCGCTGCGACCCGGGGCC
10 CGGACGCGAGTGGCTGCGGTGTCCTGGCGAGCAGTGCAGTTAGGCCGTGTCC
TCAGCTGCTTGGAACCCCTACATCCCACCATGGCTGGATACAGAAGAGGAAGTTG
ACCAGCTGGAAGAGGACGACTGCAGCTCCTCCTGTCCCTGGCGATCTCTC
CCTCTCCTCCCAGCTCTGCCTCCCTGCCCTGGACCTCTGAGGAGGAGGGACTGG
GTGATCAGCCACCCCCAGCCTGATCAGGACTCCAGTGGCATCCAGAGTTAACGCC
CATCCATCCTGAAGCGGGCTCCTGGGAGCGTCCGGTCACGTGGCCTCGATGGCA
TCACTGTCTACTATTCCCGGGTGCAGGGATTACCAAGTGTGCCAGCCATGGT
GCTGTACCCCTGGGATGGCTTCTCGTCATAGCACCTGCCCTTCTCCTTAGCCGA
GTTAACAGGAGCAGTCCGGCTGGCGTGAGAAGCTCCGTGGCGTTAAAGG
AGGAGAAGCTAGAGATGCTGAAATGGAAGCTTCAGTGTCCGGAGTCCGGAGGCA
GGGGCAGACGTGCCGCTCACAGTGGACGCCATCGATGACGCTCTGTAGAGGAGGA
20 CTTGGCAGTGGCCGTGGCAGGTGGCCGCCTGGAGGAAGCGAATTCCCTACAGCCCTA
TCCACCTCGGCAGCGACGGGCCCTACTTCGCGCTCCGGTTCGAAGGATTGACCG
AGAGGAGAAGCACGAGCTGCAGGCGTACGCCAATCCGGAGGATTGTGGTTGTC
ACTGTGATGGCGTCTGTGACCCCTGAGACCTGCAGTTGCATCCTGGCGGGATTAAAT
25 GCCAGATGGATCACACGTCCCTCCCTGTGGCTGCTGCAGCGAGGGCTGTGAGAAC
CCCATGGTCGAGTGGAAATTCAATCAGGCGAGAGTTCAGACACACACTCATCCACACGC
TCACCCGCCTGCAGATGGAGCAGGGTGGAGAGAGTTGGGGACCCGGAGTCCCCC
ATGGAGGACGTTCCCTGTCGAACAAACCGTGGTTCCCCCTTCCTCCTCCAAACCCA

CTATGAGCAATGACCTGGGGACAGCAGCTGTGGCAGCGACATGACAGACTCTTCC
ACGACCTACTCCTCTGGCGGCAGTGGCAGCCGAGCGAGGCTCCGAACCATCTTGC
CACCCCCAGCCTGCCAGGTTCCAGCTCCGGTCTGGCATAGATGAAGACAGCCTGGAA
CAGATCCTGAATTTCAGTGAACCTGACCTCGGTATTGAGGAAGAAGAGGAGGAGGG
5 AGGGAGTGTGGCAACTTGGATAACCTCAGCTGTTTCATTGGCTGACATCTTGG
TACCGGTGACCCCCGGCAGCCTGGCTAGCTGGACACACAGCCAGTTGGCTCTAGCCT
TCCATCGGGCATCCTAGATGAGAACGCCAACCTGGACGCCAGCTGCTCCTAAGCAG
CGGACTCGAAGGGTTGAGAGAAGGTAGCCTCCCCAGCAGTTCTGGTCCCCTGAGG
GGGAAGCCGCCAGAGCAGCTCCTGGACCTCAGTTATCCTCTGTGACTCCTTG
10 AGCTTCTCCAATCTGCCAGATTATAGTCTGGGGCCTCACTATACTTCCGAAGGGT
ATCTGGCAGCCTGGACAGCCTGGATTCTCATAGGCCTGTCTGAGCCGGTTACAGATGTC
AGGGATGCCAGCTCCTGGAGAGGCCAGTTGAGGACACTGCTGTGGTGCCTTGGACCT
CTGGCGCCCTCTGGAGAGGCCAGTTGAGGACACTGCTGTGGTGCCTTGGACCT
GTGCCTGTGAAGGATTGAGATGACTTTCTGCCCTGAGACCCGTGCTGCTGCTTT
TATGTGATCTGGTGTCCCCAAGGTCTGTATGTAACGGTCTCCGTGGCTGGTT
CTGCCCCCGTGCATGTGGCAATCCTCTATTTACAGTAACACTCTAGATTATT
ATTTTTATGTTTCTGTACTGAAGGGAGGGTGGAAAGGGTATCCCTTTCAATG
CCTGGCCTCTATGTCAAACAGAGGTCTCCACCTCCTACTGTATGCCTGGAGGAGG
AAGGGGCGGGTTCACATCCCCTTTCTGTACTGTAAAATGCTCCTGGTCAAAG
ACAGCTGAAAAGCAGGCCTTAGGGTTCTGTGGACCGTGGAGCTAGGTCTTCTGG
ACTCTGAAGATGTAATTATTCCTGTAATTATTTGGGACTGAGACAGCAGTGGTT
GGGCCTCTGGCAGGTGGCGGTGTTGAGGCAAAGTCTCGGTCCCCCGCCGGT
CTGGGCTTCGGTGTGGCGTGTAGGTTGAGCTGAGCAGACGGAGGCTGTGCTTGACC
ATCGGTGATCAAACCTCCCTCTGCCCTGCCAGACGCTCTAACATGCCCTCTGTCC
20 ATTTCCCTCTCCCCAAGGCCATGGTTATAAAGGCCATGTAGGATGGGAGGCCAG
AGGCCCTAAGACATGAAGCACACCCAGATCACTGTCTAGCCTTCTGGCACTG
AATCCATCCTGACCCACCACACACCCCCGGCCAGTTGGCAAGAAAGAGGTGGCTCT
TGGGGCTTTATGCCCTCATTAGCTGATGTTGGATTATGCATTATATTGT

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CTCTAAGTGTCAAGAACTATAATTATTCTCTGTGTGTGTGCCAAGAAC
GCAGGCTCTGGCCTGCCTCCTGCCAGGAGGCCTGCCAGCCTGTGTGCTTGTGA
GAACACATTGTACCTGAGCTGACAGGTACCAATAAAGACACTCTATTTTAAAAAAA
AAAAAAAAAAA-3' (SEQ ID NO:26). In addition, the L100 clone contains an ORF from
5 basepair 145 through basepair 1890. This ORF encodes a polypeptide of 582 amino acid
residues. The translational start site was assigned to the first methionine residue in the ORF. The
amino acid sequence of the L100 polypeptide is as follows: MAGIQKRKFQDQLEEDDC
SSSSLSSGDLSPPSSASPWTSEEGLGDQPPQPDQDSSGIQLTPPSILKRAPRERPGH
VAFDGITVYYFPRCQGFTSVPShGGCTLGMASRHSTCRLFSLAEFKQEQRARREKLRRR
10 LKEEKLEMWKLSVSGVPEAGADVPLTVDADDASVEEDLAVAVAGGRLEEANFLQP
YPPRQRALLRASGVRRIDREEKHELQALRQSREDCGCHCDGVCDPETCSCILAGIKCQ
MDHTSFPCGCCSEGCENPHGRVEFNQARVQTHFIHTLTRLQMEQGAESLGDPEPMEDV
PVEQTvvSPFPPSKPTMSNDLGDSSCGSDMTDSSTTYSSGGSGRSEAPNHLAHPSPLGSS
FRSGIDEDSLEQILNFSDSDLGIEEEEEEGGSVGNLDNLSCFHADIFGTGDPGSLASWTH
15 SQFGSSLPSGILDENANLDASCFLSSGLELREGSLPSSGSPEGEAAQSSSLDSLSSCDS
FELLQSLPDYSLGPHYTSRRVSGSLDSLETFHPSPSFSPRDASFLLDSLIGLSEPVTDVLA
LESQFEDTAVVPLDPVPV (SEQ ID NO:27). This amino acid sequence was found to contain
numerous cysteine residues, forming a motif that has features of a methallothionein-like motif.
Alignment analysis revealed that the L100 methallothionein-like motif exhibits higher similarity
20 with the methallothionein motif from *C. elegans* than with the methallothionein motif from
mouse.

Northern blot and *in situ* analysis using a sequence from the L100 clone revealed that
L100 mRNA is weakly expressed in wild-type rat brain. For *in situ* hybridization, Dig-labeled
cRNA probes were used as described elsewhere (Kuner et al., *Science* 283:5398 (1999)).
25 Specifically, this weak L100 mRNA expression was observed in the pyramidal cell layers as well
as the dentate gyrus of the hippocampus, thalamus, cortex, cerebellar granule cell layers, and
several fiber tracts including the fimbria hippocampus and the cingulum. In addition, Northern
blot analysis revealed that the expression of the L100 mRNA was strongly upregulated in

response to the multiple MECS treatment. Specifically, L100 mRNA expression was induced 17.2 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

The mRNA expression pattern of L100 demonstrated a compelling overlap with neuronal populations known to release Zinc into the synapse via synaptic vesicles and to take-up Zinc post-synaptically. Briefly, synaptic release and uptake of Zinc may participate in the induction and maintenance of epileptic seizures and the neuronal cell death following epileptic seizures and ischemia. The L100 metallothioneine-like motif most likely enables the L100 polypeptide to bind Zinc or other divalent cations *in vivo*. The expression of L100 mRNA in Zinc-containing neuronal populations in the brain indicates that L100 polypeptide may sequester Zinc in brain.

In addition, when acute seizures were induced by kainate treatment, the expression of L100 mRNA was strongly upregulated (Tables II and III). Kainate-induced seizures is a model used to study epilepsy. Briefly, 300-350 g male Sprague-Dawley rats were intraperitoneally injected with either 10 mg/kg body weight of kainate or PBS. RNA samples from the hippocampus, cortex, and cerebellum were prepared from treated rats at 1.5, 6, and 24 hours post-injection. This RNA then was used to measure mRNA expression by Northern blot and RT-PCR analysis. Control mRNA measurements included c-fos, GAPDH, NO-38, and ATF-4 for the Northern blot analysis, and Hsp70, c-jun, Zif268, c-fos, Clathrin, and β -actin for the RT-PCR analysis. A Phosphoimager FLA2000 (Fuji) was used to analyze the data, which was expressed as the Integral PSL - background PSL (1D evaluation with Aida version 2.0).

At six hours following kainate injection, strong upregulation of the L100 mRNA was observed, by *in situ* hybridization, in the dentate gyrus and areas CA3 and CA4 of the hippocampus as well as the associated entorhinal cortex, the cingulum, and fimbria, which are brain areas known to be highly excited in and which mediate Kainate-induced seizures. Moderate upregulation of the L100 mRNA also was found in the thalamic nuclei, temporal, parietal, frontal, medial orbital, and cingulate cortex as well as in the cerebellar granule cells. Thus, the data presented herein indicates that L100 participates in cellular mechanisms mediating kainate-induced epileptic seizures and the consequent neurodegeneration.

Table II. mRNA expression normalized to GADPH expression

	1.5 hour	1.5 hour	6 hour	6 hour	24 hour	24 hour
Clone	PBS	kainate	PBS	kainate	PBS	kainate
Hippocampus:						
L100	4622	85251	7847	15444	3940	16551
L119	2816	69982	4597	11519	2787	12944
Cortex:						
L100	-	-	81	290	86	131
L119	-	-	255	1262	538	505

Table III. Fold increase in mRNA expression upon kainate treatment

Clone	Hippocampus			Cortex		
	1.5 hour	6 hour	24 hour	1.5 hour	6 hour	24 hour
A013	9.8	-	-			
L094	3.6	-	-			
L100	18.44	1.97	4.20		3.58	1.52
L119	24.85	2.51	4.64	-		
R113	2.0	-	-			
R286	-	-	-			

In addition, when acute seizures were induced by pentylenetetrazole (PTZ) treatment, the expression of L100 mRNA was strongly upregulated (Tables IV and V). PTZ-induced seizures is a model used to study epilepsy and ischemia. Briefly, 300-350 g male Sprague-Dawley rats were intraperitoneally injected with either 50 mg/kg body weight of PTZ or PBS. Total RNA samples from the hippocampus, cortex, and cerebellum were prepared from treated rats at 20 minutes, 6 hours, and 24 hours post-injection. This RNA then was used to measure mRNA expression by Northern blot analysis. Control mRNA measurements included c-fos and

GAPDH. A Phosphoimager FLA2000 (Fuji) was used to analyze the data, which was expressed as the Integral PSL - background PSL (1D evaluation with Aida version 2.0).

Table IV. mRNA expression normalized to GADPH expression

Clone	20 min PBS	20 min PTZ	6 hour PBS	6 hour PTZ	24 hour PBS	24 hour PTZ
Hippocampus:						
L100	534	1637	854	1992	966	1903
L119	342	965	-	-	-	-
Cortex:						
L100	958	2719	1162	3740	1175	1825
L119	577	1605	-	-	-	-

Table V. Fold increase in mRNA expression upon PTZ treatment

Clone	Hippocampus			Cortex		
	20 min	6 hour	24 hour	20 min	6 hour	24 hour
L100	3.1	2.33	1.97	2.84	3.22	1.55
L119	2.82	-	-	2.78	-	-
R113	-	2.0	-			
R286	-	2.6	-			

In another study, the expression pattern of L100 and L119 was determined using two models for ischemia. Briefly, neurons degenerate in brain and spinal cord after acute insults (e.g., stroke, cardiac arrest, and trauma) and during progressive, adult-onset diseases (e.g., amyotrophic lateral sclerosis, and Alzheimer's disease). Impaired energy metabolism plays an important role in neuronal cell death after brain ischemia, and apoptosis has been implicated in cell death induced by metabolic impairment. The irreversible inhibitor of succinate

dehydrogenase in the mitochondria, 3-nitroproplonic acid (3-NP), inhibits oxidative phosphorylation and causes intracellular hypoxia. Thus, one model used to study ischemia involves intrastriatal injections of 3-NP, which is known to produce selective cell death similar to that observed in transient ischemia and Huntington's disease (McLaughlin *et al.*, *J. Neurochem* 70:2406-2415 (1998)). The other model is a global ischemic paradigm that involves a 15 minute insult by complete occlusion of the carotis.

In the 3-NP study, 220-300 g Wistar rats were intraperitoneally injected with 20 mg/kg body weight. Three hours post-injections, the brain was removed and total RNA prepared. In the global ischemia study, 220-300 g Wistar rats were received a 15 minute insult (bilateral occlusion of the Carotis/arterial pressure = 35 mm Hg). One hour later, the rats received a reperfusion followed by immediate brain dissection and total RNA preparation. Untreated rats were used as controls for each study. Ten (10) µg of total rat brain RNA (without cerebellum) was loaded per lane and blotted. Probes were prepared from the 3' untranslated regions of L100 and L119. The Northern blot data was collected using a Phosphoimager (FLA2000 Fuji, Tina software) and expressed as PSL - background.

L119 mRNA expression was upregulated 6-fold by global ischemia while L100 mRNA expression was not inducible by global ischemia (Table VI). This result indicates that only seizure related stimuli alter the expression level of L100 and that L100 is not a general marker for stress response of the cell like c-fos.

Table VI. mRNA expression after 3-NP or global ischemia treatment.

Probe	Untreated	3-NP	Global Ischemia
c-fos	18.1	26.64	216.22
GAPDH	487.02	587.51	593.31
L100	30.95	43.82	40.15
L119	55.48	41.94	332.73

Northern blot analysis using multiple tissues from rat revealed that the expression of L100 and L119 mRNA was not brain specific (Table VII). Briefly, fragments from the 3' untranslated region of L100 and other IEG clones were labeled with ^{32}P -dCTP. The denatured probe was hybridized with 10 µg total RNA from rat brain, liver, lung, muscle, intestine, eye, heart, testis, and kidney in the Quik Hyb-solution (Stratagene) at 68 °C and washed with 0.1X SSC at 60 °C. For L100, after one day of exposure, signals were detected at the 3 kb position in brain. In addition, a weaker signal was detected in heart and a faint signal detected in kidney. A strong signal was detected in testis but this signal was at a position corresponding to a size smaller than 3 kb. For L119, a strong signal was detected in heart and weaker signal in brain. In addition, only very faint signals were detected in liver, kidney, and testis.

Table VII. mRNA expression in various rat tissues.

Probe	Brain	Liver	Lung	Heart	Kidney	Muscle	Intestine	Testis	Eye
A013	(+)		(+)		(+)		(+)		
L094	+		+	(+)	+	(+)	+		
L100	+++			++	+			+++(*)	
L119	++			+++					
R113	(+)	(+)	(+)	(+)	(+)	(+)	(+)		
R286	+++	(+)	+++	(+)	+	(+)	(+)		++

(*) smaller transcript

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Another IEG nucleic acid clone was designated L111. The first round of screening

produced a clone (designated L111-5) that contained a 3.0 kb fragment of L111. A second round of screening using the coding region of L111-5 as a probe produced several additional clones.

The following nucleic acid sequence is within the L111 clone: 5'-ATTCGGCACGAGGCCAGAG

TGAAGGGGCATGGAGAAGTGGACGGCCTGGGAGCCGCAGGGCGCCGATGCGCTGCG
GCGCTTCAAGGGTTGCTGCTGGACCGCCGGCCGGCTGCACTGCCAAGTGTGCG
CCTGCGCGAAGTGGCCCAGGCTCGAGCGTCTACGGAGGCGCTCCTGGCAGCCA
ACGTAGCTGGCAGCTCTGAGCGCTGCTGGCGCCCTAGCAGCCATCGTGGGGTTAT
CACTCAGCCCCGGTCACCCTGGGAGCCTCGCTGGCGTCCGCCGTGGCTTAGGGG
TGGCCACCGCCGGAGGGCAGTCACCATCACGTCCGACCTCTCTGATCTTCTGCA
ATTCCCAGGGAGGTACGGAGGGTGCAAGAGATCGCCGCCACCTGCCAGGACCAGATG
CGCGAACTCCTGAGCTGCCCTGAGTTCTCTGTCAGTGGCAGGGCGCGGGGACCGC
CAGCTGCTGCAGAGCGGGAGGGACGCCCTCATGGCTTTACAACACTGTCTACTTC
ATCGTCTTCTTCGGCTCGCGTGGCTTCCATCCCCAGGCGTGCAGGGGGGCCACC
AAAGTCAGCCAGGCCGTGCTGAAGGCCAAGATTAGAAACTGTCTGAGAGCCTGGA
GTCCTGCACTGGTGCCCTGGATGAACCTAGTGAGCAGCTGGAATCCGGGTCCAGCT
CTGTACCAAGGCCGGCGTGGTACAACCTCAGGAACCTCCGATCTGGATGCAGC
GTTTTCTAAGAGCATCCTCTAGCTGTGGAATGTTAGATTGCAAGCCTTCTGCAGGG
CAAGGAAGTGCTACATGGCGGAGTGCAAAGGATTCAGAAGCTCTTCTGCAGGG
CATCAGTCGTAGCTCCTGTGCGAAAGACTTTACTGTGTAATCCAACTG
AGTATGTGACCCCTAACACAGTCACCTTGGGACTCCCCAAATCCTTTAGCTGCACA
CAGCTTGTCAAGACTGTCCCTCAATTAGAGTTATTGGGGTGGGGGGCTGATGGCTT
GAGTAATAGAGGTCTGGCGAGGTGTCTCCCTTTGGACCTCTATGTGTTACTAG
AATCCTGAGATTCTCAAATGTTGGTGAGAGGAGACTTTACTTTCAACTTGCTTCG
GCAGTTCCGATAACACAGGACTCCAGAATCCAGAACAGAAAGAACCTTGTGT
TTGTAGGGTGTGCAGACCCAGACGGGGCCGAGGAGCTGACTGCTCAGCTCACAC
GCAGCCAGTTATCCACTCACAGACCAACCTGGCTACTGCATAGACTGTTCCAGTG
TGGCTCAAATCCACACCTCTAGGTACCCCTGAGAAGGAAAGCCACCTGAAGAGTCA

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CTCTAATCCAACACGCTCACCCCTCACGTCCATAAGGAGCTGGCAAGGGGTG
AGATGAAGACCTGACAATTAAATGACTGTAGCATAGAGAGCCATGGCCTTGAG
TTAAGAGTCTGATCCCAGGTTCTGTCCCCACTGTCTGTGACTTAGCCACCTGT
CTGCTACAGATGGTGGTAGGAGGCCACCCTGTTGCGAAGTCCTGAGATAATGACAA
ACACAGAGGCTAGCTCACAAAATGTACTTCCTGGCCTGGCTCTGAAGGGTTAACT
GTTGGGCTCCATCCCAGATTCTGAGATCAGGAACCTCAAATATGAGGCCGCCTCT
GGCTGATTCTGATGCCCATAAATGTTAAAATGACACAGCAAAGGTTCATCTCCA
GCCAGGTGTGGTGGACACACCTGTAAGGCCAGCGCTGGAGATGGAGACAGGGGG
ACCAGTAGTTCAGGGTCATTCTGGCTACATAGCAAACCTCAAGGCCACCCTGGTCTC
AAAAACCAAAACAAAAGCCATCTCTGACTCCCTCAATTGTTCAAAGCCTTCCA
GGGCCTTCAGAACATCAGCTCAGAGTGTCTGGAAAGATTAGCCCAGAACCCAGAGA
AAGAGTACGCTGTGCTGTAAAGCCAGTTACTCTGTCCCCGTGAACCTAGGAGAC
AGAGCACTCCGACCCTATAGAGGGCAGTAGTGGCCATTCTGTAGGGACTGGTA
TAGAAGTAATGTGAACATTAAAGTTATTAAATTGCTGCCTCACATTGATT
TTATTAAACCTCACATTATTAGAAAATAAGAGTAGTAAGTGTCTGAATAGGA
AGGGAGTCTCTTAAGGCTTTCCAAGAGAGCTCAGGTTGGATTCTAGAGTCCCCC
GACCCCAAGAGAGGACTCTTAGTGTGACACGGCTTGTAAAGTAAGATGGGAGT
CCTGGAGAGAGAGACCAAGCTGATTAAACTAGGAAATGGAGTCTGAACCTGTG
GAAGATTGAAAAGTTAACGCTATGTGTCTGAAGGTACTGGCCAGAAAAGCACTT
GGCTGAAAAAGAAAACCTGTTAATTCAAGGGTGGAGGAATAGAGACAGATGAAG
AAAGCATTAGACCTCGGAAACCTGATGTCCTATGAAATTCTGTTTATAAAATTGT
GTTATGGTGGAGATCTGTCATTGACTTGTGGCTGTAAGAAACCTGTTATCTAT
GTTAAGAAAGTACTTCTAATTATTCAATGTCTCCTAAATTATCCTTAAAAAAA
AAGTTGGAAAAGTCTATGAGACCGTACCTAACGAAACCTGACTGTGATTAAAGTTAT
TTAATGCCATGCATTGTGAAGCCCTCCAGTGTGGCTGTGGTGTCTGAGGA
AATGTAAGTTGGCATGAGGGGGAGGGGCTGCTGTTCTATATTGTTTGTCT
ATAAACAGTAATCAGGATGTATCCTGGTTCATTTGACATTGAAAAAAA

ACTCGTGCAGAATTC-3' (SEQ ID NO:28). The L111-5 clone contained 0.5 kb of the 3'-end of an ORF.

Northern blot analysis using a sequence from L111 revealed the presence of a 4.0 kb mRNA transcript. This analysis also revealed that the expression of L111 mRNA was
5 marginally upregulated in response to the multiple MECS treatment.

Another nucleic acid clone was designated L117. The L117 clone is 2460 bp in length and has a nucleic acid sequence as follows: 5'-TACGGCTGCGAGAAGACGACAG
AAGGGGAGCGGAGCCAAGATGGCGCGGAGCTGGAATACGAGTCTGTGCTGTGTGT
GAAGCCCGACGTCAAGCGTCTACCGGATTCCGCCGGCCTCCAACCGCGGTTACAG
10 GGCATCTGACTGGAAGCTAGACCAGCCTGATTGGACTGGTCGCCTCCGAATCACTTC
AAAAGGGAAGATTGCCTACATCAAACCTGGAAGATAAAAGTTCAGGGAGCTCTCG
CTCAGGCGCCAGTAGAGCAGTACCCCTGGGATTGCTGTGGAGACTGTGGCCACTCCA
GCCGCTACTTGTGATCAGGATCCAGGATGGCACCGGGCGCAGTGCCTTATTGGCA
TCGGCTTCACGGACCGGGAGATGCCTCGACTTAATGTCTCCCTGCAAGATCACT
TCAAGTGGGTAAAGCAGGAAACCGAGATCTCAAAGAATCGCAGGAAATGGATAGT
CGTCCCAGTTGGATTAGGCTTCAAGGAAGGGCAAACCATCAAGCTGAGTATTGG
GAACATTACAGCCAAGAAAGGGGGTACTTCTAAGCCCCGGCCTCAGGAACGGGG
GCCTGAGCTTACTCCCACCTCCTGGAGGCAAAGTCACTATCCCCCACCCTC
CTCCGTTGCCATCAGCAACCACGTACCCACCACCCATTCAAATCTAACCATGG
AAGTAATGATTAGATATCCTGTTAGATTGGATTCTCCAGCTCCTGTCCCACCTCA
20 GCACCAAGCTCCAGCTCCAGCTTACAAGCAATGACTGTGGGGAGACTTAGCACT
GCATCCAGCTCTGGTCAAACCAACAGGCACCACAGCCATCTAACTGGGTCCAGTTGA
GTCGCATTGGCAAGAAGTTGAGGACACTTGAAGAATAAAATGACCTCAAGGGCAC
CATTCTATGAGGGAGTTGAGGGACGGCTTAATTCCCAGGACCCAAATCAGTGGTCA
25 GTCTTCCTGTAGCTCTGTGCATTCAAGGCTGGATTTTTTTTTTTTGGTT
ACCTCTGTGTTACTTGCTGTATATCCAGGAGACAATCTGCTGTTCTGCTCAGAAC
AAGCAAGGGAGTAGTGGGTATTATCACACTGACTTGCAGAGTTCAGAAGGC
CAACTTGATGAGTGGGAGTGACCTCGAACGTATGAAATCCTGAACCTTATTCAGA

ATCATCTCATGATTCCCTAGTTAGCAATTTCAGGAGAGACAAATGCCTTGAAACTGT
CTTCTCCACTAATCCGAGACTAAATATGGTCAGGCTGGCCCCAGGACTCATGAAGTT
AGGGTTTCATGGGGTAGATTGGAGAAAGCTGTGTCGGCCTCTCTGTAAGG
CCTCCTCAGGCTTACCCATGCAGTGAACCTCCCGTGTGGTGGAGCCCCATCAC
5 CTTCTGTGTTACATGTTCCCTTGACAAGAGGGTTATGTTGGTGGCACCTC
ACTGTTTCTTGTGAATAGTGCAGCATCTTGACCAGTGAATATTCTGAGATGAAG
GGGTCAAGGGCTGTGCTTCCATGGTAGTCTACAGAAGTGTAAATTCTGCG
GCCAACGGGATTGCTGCAGTGCACAGCATAGAATTGATCTACTCACCTGTGTTG
ACCTGAAGAGTTAACCTGATGTAGAGCAGAGAGCTGGAAGCACTAAGTCCC
10 TTCAGTACCCACAATGCCTGCTGCCTGGTTGACTCCTTCATAAACATTCAATT
CAGTCATCTAGCACTCTGTGGAAAGCTGCTGTTGATTGTGTCAGTGTGAAGGAGG
TGAAGTCACAGCTTCTTACCTATGACAGTTAGGCTTGCACTAGACGTTGATACCA
GCTAGGATATCTAAAGGAAGTTACCGCCCCATCACTCTCCAGTCTGGCCGCCAT
TCCTTTACAGTGCTGTGAAGAGCGTCCCTGAGGTGGTGGTACTGTCTCCTGTTG
15 GTCGGGCAGTTGAGGGAGGAGTGGGAGGACTCACACTCCTGCAGGTACCTGTTG
GGTAGCACACTGGCTGCAGAGAGTCCTTCAGATATATTGTTCTCAATGTTCTCGT
AGCTTTCTAACCTGGGTCCATTTCCCATCGCCTCTCCCATTCCAGGCAGCTC
TCTTGTGCAGAGCCATGGCAGGACGTTAAGTCCAATAAAAACACTAAGAAGAA
AGTATAGAATCACTAGTGAUTGGAAACCTATTTCTCAATCTCCTCCATTGTTG
20 TGTTCTTGTATTCTTAAGATGATAATATATTGTATTGAATTGCTGAAAATTGAA
AATGAAGAGTTGAAGATATATGTATATAAGCGTATGCTGTATTGGTCAATAATGGTAA
TTAAAGATATAAAAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAA-3' (SEQ ID NO:29). In addition, the L117 clone contains an open reading frame (ORF) from
25 basepair 42 through basepair 875. This ORF encodes a polypeptide of 278 amino acid residues.
The amino acid sequence of the L117 polypeptide is as follows: MAAELEYESVLCVKPDV
SVYRIPPRASNRGYRASDWKLDQPDWTGRLRITSKGKIAYIKLEDKVSGELFAQAPVEQ
YPGLAVETVADSSRYFVIRIQDGTGRSAFIGIGFTDRGDAFDNFNVLQDHFKWVKQETEIS

KESQEMDSRPKLDLGFKEGQTIKLSIGNITAKKGGSKPRASGTGGLSLLPPPPGGKVTP
PPSSSVAISNHVTPPPPIPKNHSNDSDILLDSPAPVPTSAPAPAPASTSNDLWGDFSTA
SSSVPNQAPQPSNWVQF (SEQ ID NO:30).

Using tblast2x algorithms, the L117 polypeptide was found to have homology with expressed sequence tags (ESTs) from mouse, mouse embryo, human hNT neurons, human tumors, drosophila, drosophila embryo, *C. elegans*, and *Arabidopsis thaliana*, a plant organism. Although the sequence of ESTs can be questionable, the identified ESTs were aligned for comparison. The comparison of consensus sequences from each species provided evidence that the L117 clone or a L117 motif has a very strong pressure for conservation during evolution since it is conserved in a variety of very distant species. In addition, this alignment indicated that the first methionine residue in the ORF of the L117 clone is the true initiation site for translation since most of the homology between the ESTs begins around this position, and the *C. elegans*, drosophila, and human hNT ESTs each contain a methionine residue that is in a very close proximity to that of the L117 clone. Further, the relation between these ESTs and the L117 clone was supported by an exactly matching stop codon in the human EST, mouse EST, and L117.

Northern blot analysis revealed that the expression of the L117 mRNA was not upregulated in response to the multiple MECS treatment in either the hippocampus or cortex. Analysis using a total RNA extract, however, revealed a small upregulation upon MECS stimulus.

Another IEG nucleic acid clone was designated L119. The L119 clone is 2900 bp in length and has a nucleic acid sequence as follows: 5'-ATTCGGCACCGAGCCAGAG
TGAAGGGGCATGGAGAAGTGGACGGCCTGGAGGCCGCAGGGCGCCGATGCGCTGCG
GCGCTTCAAGGGTTGCTGCTGGACCGCCGCCGGCTGCACTGCCAAGTGTGCG
CCTGCGCGAAGTGGCCGGAGGCTCGAGCGTCTACGGAGGCGCTCCTGGCAGCCA
ACGTAGCTGGCAGCTCTGAGCGCTGCTGGCGCCCTAGCAGCCATCGTGGGTTAT
CACTCAGCCCCGGTCACCCTGGAGCCTCGCTCGTGGCGTCCGCCGTGGCTTAGGGG
TGGCCACCGCCGGAGGGCAGTCACCATCACGTCCGACCTCTCTGATCTTGCA

ATTCCCGGGAGGTACGGAGGGTGCAAGAGATGCCGCCACCTGCCAGGACCAGATG
CGCGAACTCCTGAGCTGCCCTGAGTTCTCTGTCAGTGGCAGGGCGCGGGGACCGC
CAGCTGCTGCAGAGCGGGAGGGACGCCTCCATGGCTTTACAACACTGTCTACTTC
ATCGTCTTCTCGGCTCGCGTGGCTTCCATCCCCAGGCGTGCAGGGGGGCCACC
5 AAAGTCAGCCAGGCCGTGCTGAAGGCCAAGATTCAAACACTGTCTGAGAGCCTGGA
GTCCTGCAGCTGGTGCCCTGGATGAACCTAGTGAGCAGCTGGAATCCCGGGTCCAGCT
CTGTACCAAGGCCGGCGTGGTACAACCTCAGGAACCTCCCCTGATCTGGATGCAGC
GTTGTTTCTAACAGAGCATCCTCTAGCTGTGGAAATGTTAGATTGCAGCATCCA
CAAGGAAGTGCTACATGGCGGAGTGCAAAGGATTCAAGACTCTTCTTGAGGG
10 CATCAGTCCGTAGCTCCTGTGTGCGAAAGACTTTCACTGTGTAATCCAACTG
AGTATGTGACCTAAACAGTCACCTTGGGACTCCCCAAATCCTTTAGCTGCACA
CAGCTTGTCAAGACTGTCCCTCAATTAGAGTTATTGGGGTGGGGGGCTGATGGCTT
GAGTAATAGAGGTCTGGCGAGGTGTCTCCCTCTGGACCTCTATGTGTTACTAG
AATCCTGAGATTCTCAAATGTTGGTAGAGAGGAGACTTTACTTTCAACTTGCTTCG
GCAGTTCCGATAACACAGGACTCCAGAACAGAACAAACTGGCTACTGCATAGACTGTTCCAGTG
TTGTAGGGTGTGCAGACCCAGACGGGGCGAGGAGCTGACTGCTCAGCTCTCACAC
GCAGCCAGTTATCCACTCACAGACCAAAACCTGGCTACTGCATAGACTGTTCCAGTG
TGGCTCAAATCCACACCTCTAGGTACCCCTGAGAAGGAAAGCCACCTGAAGAGTCA
CTCTAACCCAACACGCTCACCCCTCACGTCCATAAAGGAGCTGGCAAGGGGTG
AGATGAAGACCCGTACAATTAAATGACTGTAGCATAGAGAGCCATGGCCTTGAG
TTAAGAGTCTGATCCCAGGTTCTGCCCCACTGTCCTGTGACTTAGCCACCTGT
CTTGCTACAGATGGTGGTAGGAGGCCACCCCTGTTGCGAAGTCCTGAGATAATGACAA
ACACAGAGGCTAGCTCACAAAAATGTACTCCTGGCTGGCTCTGAAGGGTTAACT
GTTGGGCTCCATCCCAGATTCTGAGATCAGGAACCTCAAATATGAGGCCGCTCT
25 GGCTGATTCTGATGCCCATAAATGTTGAAAATGACACAGCAAAGGTTCATCTCCA
GCCAGGTGTGGTGGACACACCTGTAAGGCCAGCGCTGGAGATGGAGACAGGGGG
ACCAAGTAGTCAGGGTCATTCTGGCTACATAGCAAACCTCAAGGCCACCCGGTCTC
AAAAACCAAAACAAAAAGCCATCTGACTCCCTCAATTGTTCAAAGCCTTCCA

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GGGCCTTCAGAACATCACGCTCAGAGTGTCTGGAAAGATTAGCCCAGAACAGAGA
AAGAGTACGCTGTGCTGTAAAGCCAGTTACTCTGTCCCCGTGAACTAGGAGAC
AGAGCACTCCGACCCTATAGAGGGCAGTAGTGGCCATTCTGTAGGGACTGGTA
TAGAAGTAATGTGAACATTAAAAATAGTTATTAAATTGCTGCCTCACATTGATT
TTATTAAACCTTCACATTATTAGAAAATAAGAGTAGTAAGTGTCTGAATAGGA
AGGGAGTCTCTTAAGGCTCTTCCAAGAGAGCTCAGGTTGGATTCTAGAGTCCCCC
GACCCAGAGAGGACTCTTAGTGTGACACGGCTTGTAAGTAAGATGGGAGT
CCTGGAGAGAGAGACCAAGCTGATTTAAACTAGGAAATGGAGTCTGAACGTG
GAAGATTGAAAAGTTAACGCCTATGTGTCTGAAGGTACTTGGCCAGAAAAGCACT
GGCTGAAAAAGAAAACCTGTTAACATTAGGGTGGAGGAATAGAGACAGATGAAG
AAAGCATTAGACCTCGGAAACCTGATGTCCTATGAAATTCTGTTTATAAAATTGT
GTTATGGTGGAGATCTGTTGCATTGACTTGTGGCTGTAAGAAACCTGTTATCTAT
GTTAACAGAAAGTACTTCTAATTATTCAATGTCTCCTAAATTATCCTTAAAAAAA
AAGTTGGAAAGTCTATGAGACCGTACCTAACGAAACCTGACTGTGATTAAAGTT
TTAATGCCATGCATTGTGAAGCCCTCCAGTGATGGCTGTGGTGTCTGAGGA
AATGTAAGTTGGCATGAGGGGGAGGGGCTGCTGTTCTATATTGTTTGTGAGGA
ATAAACAGTAATCAGGATGTATCCTGGTTCATTTGACATTGAAAAAAA
A-3' (SEQ ID NO:31). In addition, the L119 clone contains an ORF from basepair 28 through
basepair 768. This ORF encodes a polypeptide of 247 amino acid residues. The translational
start site was assigned to the first methionine residue in the ORF. The amino acid sequence of
the L119 polypeptide is as follows: MEKWTAWEPQGADALRRFQGLLLDRRGRLH
CQLRLREVARRLERLRRSLAANVAGSSLSAAGALAAIVGLSLSPTVLGASLVASAVG
LGVATAGGAVTITSDLSLIFCNSREVRRVQEIAATCQDQMRELLSCLEFFCQWQGRGDR
QLLQSGRDASMALYNSVYFIVFFGSRGFLIPRRAEGATKVSQAVLKAKIQKLSESLESCT
GALDELSEQLESRVQLCTKAGRGHNLRNSPDL DAALFF (SEQ ID NO:32). Hydropathy
plot analysis revealed a stretch of about 50 hydrophobic amino acid residues, possibly indicating
that the L119 polypeptide is a type II transmembrane protein.

Northern blot analysis using a sequence from the L119 clone revealed that the expression of the L119 mRNA was strongly upregulated in response to the multiple MECS treatment. Specifically, L119 mRNA expression was induced 17.8 fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I).

Another IEG nucleic acid clone was designated R010. The R010 clone is 1280 bp in length and has the following nucleic acid sequence: 5'-GCTTGAAACCGGACTGCAGGCTAAACTGGCTTGAATCCTGGAAGCATAAAGGACAAGTAGCAGGGCTCGCAGTCTTCCATTGTCAGGGAGAAGAACTTATAATTCAAAGATCTGGGTCTGGACCCAGGCTGACCACTTGGAGCTTGAGACTCTGGGATTGTGATCCAGTTCTGAGCTGGTGTAAACACTCCTGTGACTTTGGTCAATTCTAGCTACCAAGATTCCAGCCAACATGACCTCTGCAGCCTATAAGGAGAAGATGAAGGAACCCCCACTAGTGTCTGTTCTGCTCTGTTCTGTGATCCCCTGAATAATCATCCTACAAATATGAAGGCTGGTGTGGAGACAGTGTAGGAGGAAAGGTCAAAGCCAGCGGAAAGGCAGTGCTGACTGGAGAGAAAGAAGAGAACAGGCAGATACGGTAGACCTGAACCTGGTGTGTCATCTCTGATATGGAAAGTCATCGAGCTGAATAAGTGTACCTCGGGCCAGTCCTTGAAGTCATCCTGAAGCCACCTCCTTGACGGGTGCCTGAGTTAATGCCTCCCTCCAAGACGTCGAGACCCATCGCTAGAAGAGATACAGAAGAACGCTAGAAGCAGCAGAGGAGCGAAGGAAGTACCAAGGAAAGCTGAGCTCCTAAAACACCTTGCAGAGAAACGAGAGCATGAGCGTGAGGTAATCCAGAAAGCTATCGAGGAAAACAACAACTTCATCAAGATGGCGAAAGAGAA GCTGGCCCAGAAGATGGAGTCCAATAAGGAAAACCGGGAGGCCATCTGGCTGCCATGTTGGAGCGCTGCAAGAGAAGGACAAGCACGCAGAGGAGGTGCGGAAAAACAA GGAGCTGAAGGAAGAGGCCTCCAGGTAAAGCCANAGGCCAAGGAAGTTCCAGGACAGCTCCCGCAGCAACCTGGTCCAGCAGCATGGCCGCTGGCTGCTCTCCAGCAACTGGGTTGGGGGGAGGGGGTGGCAAAGGGCGTTCCCTGCTTTGGTGTACATGAAAAGATTGACCAAGTGAAGCCATCCTATTGTTCTGGGGAA CAATGATGGGGTGGGAGAGGGGACAGAGAGGTGTTGGAAAAGGAGGTGAAGATGACCCGAGACTTGTGACACTGTCCACTGACTGCAGACTTGGCCAAGGCCCGCTTTCACGGCTCTGCCTGGACATTGGCCTCCAGGTTCTAGTGGAGAGAAGATGTGA

CAGAAGTCAGAGTGAAGGGCCGAGTCCTGGTGGGTGGTGCAGGCCAGCAGG
ACGAGCCCGTCTGGATGGAGTGAAACCTACCCCTGAGCGGGTGGGATAAGGTCTGTG
TGCCTCTGTTCATTCATCTTGATCATGACCAACGAAACATTAAAAAAA
AAAAAAAAAAAAA-3' (SEQ ID NO:33). Two genomic R010 clones were also obtained.

5 The nucleic acid sequence for these genomic R010 clones is as follows: 5'-GATAA

ACACTCCTGTGACTTTGGTCAATT CAGCTACCAGATTCCAGCCAACATGACCCTCG
CAGGTAGGTACATGCACCAGTCAGTGATGAACACCATAACACAAGCCATTTCAT
CTCTGTGTGTCCATGTGTATTAAAGGTGCATCCGTGTGTGATACACACGTAGGT
GCATGGCATGCATGTGTGCAAATGCATATACAAGTCCAAGGACAGGGTTGGGG
10 ATTAGCTCANTGGTAGAGCACTGCCTANGAACGCAAGGCCCTGGGTCGGTCCC
CAGCTCCGAAAAAAAAGAACCAAAAAAAAAAAAAAAAATTTCCAN
GGACAACCCAAATTCCNTTCNCNAANCCANCCANCTCCATTNAAAAAAANG
GGTCNCNCNTGGGTTAAACCATTNNAAANGCNAACCTNACNGGCCAKTGAKTGC
CAGGAATCTTCTTATYCCTGCCWACCTCCAATGTCTTCACATGTGAATGCTGAGG
15 GTCAGAACTTGTGCTTACAAGGCAGACATTGCCAGCTCTCCGCCATCTTCTCA
TGTATGTACACTCACAGATGCACAGGAAGAGAGGGTAGAGAACGCAAGAGGCAA
GTCATTCTGGTGGTGGGATCACAGCTGAATTCTCTCCTCATTTGCTCTGT
20 GTGTATTATTAATTAAAATAACCTTATAATAGTATCGAAACTATGCTTCAA
GTTGTAAGAGAAAGTGATCACTGGCTGTGTAGTGAGGGGTCTTATATTATGCA
TATAACATGGTGCAATGGGAAGGACTGGCAGAGGCCTCCATGATGACCTATGACTTC
TAGGGAGACTCAGTCGTCAAGGGTACATTCTACTCTGCAGACAGCTCTCCCTG
25 GTTGATTCTGTGCTGGGAAGATTGAGGAGTCTCCAGCCTGACCTCTACAGT
GGGCCTGGACTTAAGGAGAGTAGCAAGGAAGTCTTTATTAATCTCTTACCCCTT
AGGCAGCAGTGTCAAGTACTTTAGCAGAATTAAATAGATTCTACAAACTACA
AACTCAAAGCCCTGGTTATCCTGGTGGGAGTAGGAGATGGAGGGCCAGGGTC
AGGGCACTGCACCTGGGATCTTACTTGAGGGTACTCAACGCTTGGTAGTAACAAAA
AGTGGGGTGAGTGACAATGTTAATTCTAACTGGGAGGTAGCCCAGGCTGGTACT
TTGGAGCCAGAAAGCCTGGCTGACTCACAGAAGTGGTGTCTCTYGYAGCCTAT

AAGGAGWWGATGAAGGAACTCCCACTAGTGTCTGTTCTGCTCCTGTTCTGTCT
GATCCCCYGRATAAATCATCCTACAAATATGAAGGTGAGTAGGGCTAGGCTGGGA
TAGAAAAGGGTGGAGGCTCTGTGCCTGTGTTGTSGGTCCCCACATTGACTCCTA
TCTTGTAAAACGTGCCTGGTCGCAGTGTCTTATTCCCAGAGGCTGAGGAGTCTG
5 AGCCCAGGGGATGTAGCCTGGGTGCCAACAGCAGCCTCCAGGGATCTGGATTGGGCC
CTCCTGGAGCACTGCTCCTAGAGTCCCTTRCACATTGACACCACAGAGGAC
ACCAGGATAAGCCAGACACAAGTTGAGATTCCATTGAGGCCCCAGAACAGA
AAAAGAAAACCTAGTGTGTTACCAGGGCTCTAGGGACAGGTAGAGATGCTCCTA
10 GACAGGTCCAGGGTGGGAATAGCACTTAACCTGGATGGTGACAGTCAGCCCT
AGACCCTATCAGAGAGTACTGGATTGTCATGCTGTCAGGAGGAGTGGTCAGGGAC
AGATAGGTCACTCTTCATTCTGTTGCCAGGAAGGGATGGGTTGGTCTGTCAATA
AGAGAGATGGGTGTTGGATGACCTGAGTCTGTTTTCCATTAGGCTGGTGTGGG
AGACAGTGTAGGAGGAAAGGTCAAAGCCAGCGGAAAGGCAGTGACTGGAGAG
AAAGAAGAGAACAGGGTAGGCCGGAGCCAGGGAGAGGTCCACAAGCCATCAGAG
GGACAGGGCAAGGAGGGCTGGCGGTGGGATGGTGAAATGAACGGTCTGTC
15 ACCAGCGAGGAACAACAGCAGCTGGTGTATCACAAATCACAGCTCCCTGCTTACCC
TGTAAAAGCCATTGACCTAGGGCCAACGTTCAAGGATCGACCAGACCCCTAGTCAT
TGGTGTGCCTTGGGACCCCTCAGCTTCTGTGTGATGTACACATGCTCATT
GGGGCCCCAGCTGCTCCTCAGAAGGTGAGCAGCCCCAACTCTGCCCTCCATAGCAGA
TACGGTAGACCTGAACGGTGTGTCATCTGATATGGAAGTCATCGAGCTGAGTAA
20 GTGTACCTCGGGCCAGTCCTTGAAGTCATCCTGAAGCCACCTCCTTGACGGG
GCCTGAGTTAAAGCCTCCCTCCAAGACGTCGAGACCCATCGCTAGAACAGATA
GAAGAACGCTAGAACGAGCAGAGGAGCGAAGGAAGGTTAGTGTAGCCCCATGTC
ACTTCCTCCATCCCAGCGGGAGCAGGAAGTCAGCTCCATATCTCTCCATCCC
25 GTGGGAGTGGGAAGGATATTAGACAGCACCTGAGTGCTGGCATAGACCGGT
AGTTCTCAACCTCTTAGTGCTGTAACCCCTAATATATATATATATATATAT
ATATATATATATAGTCCTCATGTTGATTACCCCCCATACCATAAAACTATCCC
TGCTCTTATGTCTTCATAATTATAATTGCTACTGTTATGAATTGTGATACA
ACTAT

CAGACCTGCACCCCTAATGGCAGCAGCCCACGTGTTGAGAACCACTGGCATAGAT
GTAGACTAAGATAACCACCTGAAGGGGACAAGACTATGACTATGCACTGGTGAGCT
TACAGTGTGGCTAATGGCTAAATGTCACAGTCCTCACAAAGCTGCCCTGTATGCA
GCTTCCTGTTCCCCATTGATTCTMGTCCSTCAGCTCAGATGCCATTAAATGTGAG
5 TGTTCTTNACCTTCAGAAANACAAAACAAAACAACCCAGCTTCTCCACTNAATT
GTGTGGTCCCTCCCTTAAATATCAAAGCATTATCACACCCAGGTCTGGNGTCCA
NTATNTATTGATATGCGTGTATTNNACTAGGGCAATTNTCTCCNTCCCTGGTGT
CTGGAGTTGTGAGGGCCTTGAGGTTATAGAAGATCACTTAGTACTTGTGAATGAAC
GCGAGGAAAAGGAGAAAAGAGACTCAGAAGCTACTNNGAAAGGGTACNAAAGC
10 CAAATATGACGGAAAGGTTGCAGTCATGNCGTTCTGCTTCTGGACAGAG
GACCAGGTTCATCTCATCTGGCATGGCACTGTCAGCTGTGGTAGAAATCCAC
TCTAAAGGGTCNTCTCTTCTTGTGNTGCCCTAGTACCAAGGAAGCTGAGCTCCTAAA
ACACCTTGCAGAGAAACGAGAGCATGAGCGTGAGGTAATCCAGAAAGCTATCGAGG
AAAACAACAACCTCATCAAGATGGCGAAAGAGAAGCTGGCCCAGAAGATGGAGTCC
AATAAGGAAAACCGGGAGGCCATCTGGCTGCCATGTTGGAGCGGCTGCAAGAGAA
GGTAAGAGGTCTGGATTGGCAGGAGGCTCCTCCATGGCAAGAACGTGCAACCTA
CACATCACTCTGGAGGAAGCGGCCTATGCAGGAATTGAAATGTTCTACCAGGCAG
GGTCCTCATTGTTCAAGGGGAAGATTGGGAAGTCATAGGCAAGAACGCTCACACC
AAACCCCTGGGTGGCCTCCGGGATCTTCTANGTTGAACCGGAAATTCTGCACTG
TCTCANGAGCTGCTCACACCCCTTTCTAAAGAAAGCCGCCAGTGCAAGTAT
20 CTAAGGAGAGGCACATGTCTACACATTCTGGCTCATCATTGAATGGCAGATTG
GGTAGTGAAAGATAACAGTCAGCTGGCTTGAGCCANGGATACAGCAAGCTCGGTT
GCCAATACAGCAGGATACAGGATTCTCCCCAGAGCTCCTCGTAAGGGCCAGAGAGT
ANTAGGTTTCTCAATAGTCTGCCTTGTCAATAACTCAAATGTCACCTGCATCTGA
GCGGTGTGCGAGACTGGGTTGGCCTCCATGTTATTCTTGAAGACGTGCTGACC
25 TCATTCTGAGTCCCAGGCTGCCTACGTTCTCCTGCAGCTCCTGGGAAGCTTAC
TCTGTGTTTATTCCAAGGAGCCGCTGCTGCGCGGTGACTCCGGGACSGATCGGT
GGCCTCGTCCCATGGTGAGCAGCGTGGCCTTATTCTCCTGCCTACCCACCTAAAA

CCTCAGGCCCTGACAATTACCACAGAAAGATCTGGCTCATCCAGGGATGTGAGCA
GCACAGGCTGCCAGTAGGTGGCAGCCCTGTGCTCATGTTCAATTACAGGAGGGAC
AGCAAGGCTTCTTCTCCACTGAGTGCCTGGGGAGGGACACAATCTGAGTGTGAC
TTTGGGCTCCTCCAGTTAATGAGAGATACTGTAAGAAAACCTTAAGATTGCCTTACT
5 TTTTATACCAGGTCTCATGCATTCCAGGCTGCCCAAATTGGCTAAATTGCTGAGG
CTAGCCTTCCAAGCCTATTCAATGCAGGTTGGGCTGAACCCAGGGCTGTGCATG
GTGCTGCCAAGCCTATTCAATGCAGGTTGGGCTGAACCCAGGGCTGTGCATG
CAAGCTAGGCACTCTGCCAACAGTGCCTAGCCCCAACTCAAGGCAAATTCTGAGG
AAACCACAGATAGAATGGGAGAGTTATGGGATTGCAGACTCAGCTAAAATACATC
10 ACAAAAGTTAGGTTGTGAAGCACTTGAATGTTGTTATATAACGATTCTATTAA
TCATAACTCGGTATCACAAGTTACAAGGCAAACATTCTAGTCCAGATAAGGAAA
CCATTCTAGAGGTCAAATGATTCCAGAGATTNACAGGGTATACGACAATANATTGGC
CCTGGCCNCTAATCAATGGCTGCTTCTGCCGGTAAAGAAAACATCCAATATAANC
CACNNCTTCANAGCAANAATTCAAAGACAACAAGCAGGGCAAAACCAGGGTCCA
15 AAGCAACCACT-3' (SEQ ID NO:34) and 5'-TGGGCGGGAAAGCAG
TTTGTCTTGTGNTGAATTATGTTANNAAGCAAATGAAGTTATCTTCCAACACATGTG
AGGGAGTCCATTGTCTGGAGTCAGCANTATTCCAACAGTTCTGTCACTACAT
AACGCAAGGTCCCTTCAGTCAGAGATTAAAGACAACACTAAAGAGATGGAGAGA
AATAACACATCTGTGGTGTGTCAGGGACGCTGGCAATGGGCTGATCTTCCCATT
20 NTTNTAAACTGGCTGTCCAAAGGGCCNTGTATTAGTCAAGTGACCATTCCAAG
CGCCAGAATGACCAGTGGAGGTGCAGAGAGCNTAGGGTGTCTGGGTCGCTGTGA
GGTGGGTCCCCTGCAGGATGTCTATGCACCTGCAGGCTTACACCTGTGCCCCGCG
TNTTACTTGCCTCCTCCACCCCTCTTAGGATACCTCGCCGACAGCTCTGCTCTGCC
CGTGGTGACCATCTTGCCTCCATTCTCTGCCCTTGTCTCCCTGGCAGCCTTG
25 TGTGACCCGCCTTGTCCCTCCCTCCAGGACAAGCACGCAGAGGAGGTGCG
GAAAAAACAGGAGCTGAAGGAAGAGGCCCTCAGGTAAAGCCCAGAGGCCAAGGAA
GTTTCCAGGACAGCCGGACAGCTCCCGCAGCAACCTGGTCCAGCAGCATCGGCTGC
TGGCTGCTCTCCAGCACTGGGTTGGGGGAGGGGGTGGCAAAGGGCGTT

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CCTCTGCTTTGGTGTACATGAAAAGATTGACCTGTGA-3' (SEQ ID NO:35). In addition, the R010 clone contains an ORF from basepair 80 through basepair 727. This ORF encodes a polypeptide of 216 amino acid residues. The translational start site was assigned to the first methionine residue in the ORF. The amino acid sequence of the R010 polypeptide is as follows: MTAAAYKEKMKELPLVSLFCSCFLSDPLNKSSYKYEGWCGRQCRR
KGQSQRKGSADWRERREQADTVDLNWCVISDMEVIENKCTSGQSFEVILKPPSFDPGV
EFNASLPRRRDPSLEIQQKLEAAEERRKYQEAELLKHLAEKREHEREVIQKAIEENNFI
KMAKEKLAQKMESNK ENREAHLAAMLERLQEKDHAEEVRKNKELKEEASR (SEQ ID NO:36). The R010 clone was found to have homology to the stathmin family of polypeptide,
including stathmin, SCG10, and XB-3. In addition, the R010 polypeptide was found to contain a unique 27 amino acid sequence (encoded by exon 3) that is alternatively spliced to lead to the formation of two distinct mRNA transcripts.

Northern blot analysis using a sequence from the R010 clone revealed that the expression of L119 mRNA was restricted to brain. In addition, R010 expression was found to be developmentally regulated. Further, R010 expression was found to be rapidly induced *in vivo* in the dentate gyrus in response to the multiple MECS treatment and LTP stimulation, and rapidly induced *in vitro* by NGF treatment of PC12 cells.

Another IEG nucleic acid clone was designated R042. The R042 clone is 3978 bp in length and has a nucleic acid sequence as follows: 5'-CGCGATGGCGGCCGGCTGCT
GTGGTGGCAGCGACGGTCCCCGCGCAGTCGATGGCGCGACGGCGCGTCCTCCGT
GCACTGGTCCGCAAAGGACTACGGCTCACGACAACCCCGCGCTGTTAGCTGCCGT
GCGCGGGCGCGCTGTGCGCTCGTACATCCTCGACCCGTGGTTCGCGGCCCTC
CTCGTCAGTGGGCATCAACCGATGGAGGTTCTACTGCAGTCTCTAGAAGATCTGGA
CACAAAGCTTAAGAAAGCTGAATTCCCGTCTGTTGAGTCCGGGTCAGCCAGCTGA
TGTGTTCCAAGGCTTTCAAGGAATGGGGGTGACCCGCTTGACCTTGAATATGA
CTCCGAACCCTTGGAAAGAACGGGATGCAGCCATTATGAAGATGGCCAAGGAGG
CGGGTGTGGAGGTGGTACTGAGAACTCTCACACCCTTATGACTTAGACAGAATCA
TCGAACTGAATGGCAGAAACCACCCCTACCTACAAGCGCTTCAGGCTCTCATCA

GCCGTATGGAGCTGCCAAGAACGCCAGTGGGGCTGTGAGCAGCCAGCATATGGAG
AACTGCAGAGCTGAGATCCAGGAGAACCATGATGACACACTATGGCGTGCCTCCTTA
GAGGAACTGGATTCCCCACAGAAGGACTTGGCCCAGCTGTTGGCAAGGAGGAGA
GACAGAAGCTCTGGCCCGCCTGGATAAGCACTTGGAACCGAACGGCCTGGGTTGCCA
5 ACTATGAGAGACCTCGGATGAATGCCAATTCTTGCTGGCCAGCCCCACAGGCCTCA
GCCCTACCTGCGCTTGGCTGCCTCTGCCCTCTTACTACCGCCTGTGGGA
CTTGTACAGAAAGGTGAAGAGGAACAGCACACCCCCCTCTCCTTATTGGACAAC
CCTATGGCGAGAATTCTCTATACAGCGGCCACCAACAAACCCCCAGGTTGACCGAAT
GGAGGGGAACCCATCTGCATCCAGATCCCCTGGACCAGAACCCCCGAAGCCCTGG
10 CCAAGTGGGCCAGGGCAAGACAGGCTCCCTGGATTGACGCCATCATGACCAA
CTGAGGCAGGAGGGCTGGATCCACCACCTGGCCCGCACGCTGTGGCTGCTCCTC
ACCCGAGGGGACCTCTGGGTCAAGCTGGAGAGCGGGGTCCGGTATTGATGAGTT
GCTCCTGGATGCAGATTCACTGCAGCTGGATGTGGCTGTCCTGCAG
TGCTTCTCCAACAGTTCTCACTGCTACTGCCCTGTGGCTTGGCCACGCACG
15 GACCCCCAGTGGGACTACATCCGGCGATACTGCCCAAACGTAAAGGCTCCCTCT
CGATATATCTATGAGCCCTGGAATGCTCCGAGTCGGTCAGAACGCCGCTAAGTGC
ATCATTGGCGTGGACTACCCACGGCCATCGTAACCACGCAGAGACTAGTCGGCTC
AACATTGAGCGGATGAAGCAGATCTACCAACAGCTGTACGATAACGGGGCTTG
TCTGTTGGCATCTGCTCCCTCTGTGAGAACACCTCAGTCACCCTGTGGCAGAGCCT
20 GGTTCTAGCCAGGCTGGAGCATCAGCAACACAGGCCAGACCACTGTCCAGTGG
CCCAGCCTCCCCAAACGCAAGCTGGAAGCAGCTGAGGAACCTCCAGGTGAAGAAC
TGAGCAAGCGGGTAGAGTGACAGTGACTCAGATGCCTGCCAGGAGCCACCAAGC
AAGGACTCCTGAGACTGGAGAGCCATTGCTCCGTGAGCAAAGCCAGGTGCCTGAG
CTGCCATGGCCACAGAGAACATGGAACCTACAGAGAACAGTCACCAACAGAC
25 AGAGCGAGCGACTGTGTGTGCAGAGGGAGGTGTGGTGTGCCGTTGCGTGTGCAT
GCATCTGTTACACTCTCATGATCCTGAATGTTGCCTGTGCTGGAGGAGCCCTAGAT
CATGCCTTCTTACCAAGGGCTTTCTTGACTCCAGACATAAGACTAGAACCGCAG
CAGTAACCGTCAGCCAAATCTGCCCTGGAGCCCCAATAGGGTGGTAAGACCCT

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AGCTTGAATTCTGGTCTCTGCCTCCCCAGACTCTTCTCCTCCCTCTTTAACAGG
AGCTGGAGGGCCACATTTGACTCTCATCTAAAGCATGGAGTTCAGAGGCAGTCA
GAGTCCTGCTGACTTAGTCCCACTTCTGACACTAGAACCTGAGCAGGCTGGAAT
AGATGTGTCCTGTTGATCTAAACAGCCTGCCAGTCTTCTATAAAAATCCTGTGCCA
TTAACAGGCTCCCTGATGTCTAAGGCTACAGACTAGTGTGTTGTGCCAGTACT
GCTTATGTCAGCCTCAGACATAATATCAGTCTTGAGAACCTCTAAAAAAACCA
CATGGGAAATAGACTCCCAGTCTCTGTCCCTCCCTAGCAGCTAAGGTCCAGTCTC
GACCTCTAGAACGCTGTGGACAGGGCTAGGGCTGAACGTGAAAGAAACCCAGGT
CCCACAGCTGCAGGGCCCTGGTCCCTGGCTGTACTCCTGACACACCACATGCTCCA
GCCAGTACTGCTGATATCCAGCCAGGCAAGCTGGACAGCCTGGCTGGTCAGCACCTG
CCCTGCAGTGTCACTGCCAGGACTGAGCTCCGGAGACTCAGACAGACTTAGGG
GTGGAGCACTGCCTCTGGCAGTTGGCGAGAGGTAGAGACCATGCCTGGCACATCA
ACATCTCGCAGAGCAGCAGTGAAGGATTGACATAGAGAAGTCAAGCCTGCTTCC
AGGGGAGCCAACCTCCCTCCACTGTTGGGTATGGAGAAAGAAGTTATGAAA
GGATCTGGGGTACCTGAGCAAGTCTCCTCCACCCCGTGGCTGCATTGAGCCA
CAGT
GTGTGTGTAG
AGAGAGAGAGAGAGTTGTTCTGTTGGATTTGTTCTCACATGTAACATTAAGCTGG
CCTCTGGGCCTTCCCTACCTCCCTGTGACCTTCCTAGCCTCAGAGTTGAA
TGCCTTGGCCCTGGCCTTTTGTGTCAAGACAGAACCTGGGTCAAGGCTCCCC
CCTCCAGCTGTCTAGCACATCTGACAGGCTTCTTGTGAGATGGCCTCAGGTTCTC
AGCAGAGAGCTGCCCTAGTCCAAGTCTTATGTTCATCATCCTGACTAGAACATC
CTACGATTGTGTGAAGAACGGCATCTGTGATGCCATGTTCAAGAGTCATGGGTGTG
GCCTCCCTGTCCCTAGCCCCAGGCCAAGAGGAAGGGCAAAGGGCTTGTGGAG
GGACAGTAGAATGCGTCTGGAGAACTGGTCCCAGAGGAGCAAAGGCTTATTCTGGG
GCCAGTATTATTGCAACATCTCAGCTATGGGACAATGGCCTCTGTCTT
TGATGATGGCTCTCCTCAAGGTACAAGTTGGCAAGGTATCTGCTTCCACCTCC
TTGACATGTTGGCCCATTCCAGGACAGCCTCCAGTGAATGGAGCAGACTATTCCA

CAGCTGTGGGATAGAGTGTCTGGAGCCCTGGAATGACTCATGCCTCCTTGCCT
AGCCTGAGTGGCCCTGAGGACTGTCACAGAACAGTGCCCCATGTCCTGCTCCTGGC
CCGAGCATGGGAAGAGATGGTTGCAGGCAAGAGCACTTACAGCATTCCCCATTG

CTGGGAAGGTTGTTCTCCTACAGTGTGAATACTTACCTGTTATAAAATGTCTGA

5 TCCTGTCTGAGTAAAAAAAAAAAAAAA-3' (SEQ ID NO:37). In addition, the R042 clone contains an ORF from basepair 51 through basepair 1790. This ORF encodes a polypeptide of 580 amino acid residues. The amino acid sequence of the R042 polypeptide is as follows:

MGADGASSVHWFRKGLRLHDNPALLAAVRGARCVRCVY

ILDPWFAASSSVGINWRFLLQSLEDLDTSRKLNRSRLFVVRGQPADVFPRLFKEWGVT

10 LTFEYDSEPGKERDAAIMKMAKEAGVEVVTENSHTLYDLDRIELNGQKPPLTYKRFQ

ALISRMELPKKPVGAVSSQHMENCRAEIQENHDDTYGVPSLEELGFPT EGLGPAVWQGG

ETEALARLDKHLERKAWVANYERPRMNANSLLASPTGLSPYLRFGCLSCR LFYYRLWD

LYRKVKRNSTPPLSLFGQLLWREFFYTAATNNPRFDRMEGNPICIQIPWDRNPEALAKW

AEGKTGFPWIDAIMTQLRQEGLWIHLARHAVACFLTRGDLWVSWE SGVRVFDELLDA

DFSVNAGSWMWLSCSAFFQQFFHCYCPVGFGRRTDPSGDYIRRYLPKLKGFPSRYIYEP

WNAPESVQKAACKIIGVDYPRPIVNHAETSRLNIERMKQIYQQLSRYRGLCLLASVPSCV

EDLSHPVAEPGSSQAGSISNTGPRPLSSGPASPKRKLEAAEPPGEELSKRARVTVTQMPA

QEPPSKDS (SEQ ID NO:38). The R042 clone was found to be a photolyase receptor based on

sequence alignment data. In fact, the R042 clone was found to be the rat paralog of human and mouse clones based on the following observation. The identity between the human and the mouse clones is considerably higher (97%) than between either the human clone and R042

(72%) or the mouse clone and R042 (71%). This lack of a higher identity between the mouse

clone and the rat R042 clone is more than that expected from species-to-species differences.

Thus, the R042 clone most likely is a different member of the family of photolyase/blue-light

receptor homologues. The translational start site was assigned to the second methionine residue

from the 5' end based on the alignment data using the human and mouse members of the

photolyase/blue-light receptor family.

The R042 clone potentially has two differentially spliced forms at the 3'-end. The difference between these two forms is 142 bp. The shorter form was found in four clones while the longer form was found in one clone.

Northern blot analysis using a sequence from the R042 clone revealed that the expression of the R042 mRNA was strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R053. The primary library screen produced 40 positive signals that were isolated. The following nucleic acid sequence is within the R053 clone: 5'-TTGGCACACAAAGTCTGTCTCAGGACAGCTGATCCATTACTTA CRAATTCAAGAAAGTAAACATTGGCAGTATGGATCTGGTTACTTCATGGTAAGTGCTC TAGAATTACGCCAAGGCCATCTCTTGCCTCACTGTTAGTGACCGGAGTAAAGC ATGGGGCCACTGAAACTCCACTTACAATTGGGCTTCTAAATTAAAGGAAAAATT TTGATTTAACCAACACTGGATTCCAAGTTCATCTTATTCYAAATTAGGCCACTGA GCCTGTGATGTTTGGAAATATATGATTAGTCCACTGGTTCACTGGATGTTACCTATC ATGTTATGTAGAGAACAGCCATAACTATTGGTCACGATGTCGTCCTCCGAATTGGG AATGGCTCTGTTGGAAACAAAGTATTGTAAACACGTTGATCAAAGCGGTGTGC TTTGGCCTTCCGGAAATCACTGATTATGTTGAAAACCTCCTTAATTGTATTTGCA ATAAGCTATTNTCCCTNTNATGNCTGCCATGCTCCTGCTTGCAGTGTGGTCG CATGCCATCNGCTGGTAACCCANGATGGCTGCTGCNCTGATATNCACCATGCNAA ATACCACTTCT-3' (SEQ ID NO:39).

Northern blot analysis using a sequence from the R053 clone revealed the presence of a 4.9 kb mRNA transcript. In addition, this analysis revealed that the expression of the R053 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R055. The first library screen produced a clone designated R055-7 having a 1.7 kb fragment. A second library screening using the 5'-end of the R055-7 as a probe produced several additional clones having fragments of about 3.0 kb. The following nucleic acid sequence is within the R055 clone: 5'-TGAATTGCAGTAAC AGCCTTGCCCTTCTATTCTGTAGAAATGACAGGGCTTCACAATCCTCACCAAGTGGC TACTAAGCTATAATTAGCTGAATAGAAAGAATGTGGAAGTGGTCTGAGGCATATAG

AGCATATGCCAAGAACACTACCATAATGGCATCAGCTTGGTACCAAGAGAAATT
TCTTAGTCATTAGACCATAACAGTAATATATCATATGTAAATCTTAGATTCAAT
TTGAGAACCTCCAAAAAAAAGGAGCAAAGAACATGCATAAGCTATGTGTTGGCAAAA
GTAATTATATTAAAATTGACCTGCCTTGTAAAGATTAAGTGGTAAATGTCATAGT
5 GGTGGGTTTACGTCTAACCAATCTCTGAGGTTATTCTCCTGCAGGGATGGTT
CATGGCCTCTCTCCCGCTGTAGGAAGATAGCAGAAGGATGAGGATTAATTGTAGCA
TTCACTGATCCTCGTCCCAGGGACTAGGGACAATAGAAATCTGCAAACATGGAGA
GTCTGTATAAATATTGCTTTGAAGGTGTTGGCTTGATTCTGTAGAAA
ATGGCATTATACAAATTATGGGAGCAACCAACTTCTGTTCTGTTTGAAGTGCT
10 ACTATGAACCATTCAAGAGTCGTATTTTTTTAAAATTGGCCAGATATCCCCA
GCTAATGAAAAATAG:TCACCATTCTTGAAAAAGTGGAAAGCTAGAACCCCCAATT
CAAATTATTGTTGAAGATGTTCTCAGGCTACTGTATATAGAAATAATGTTTTAAG
AAAAATCAAAGAGAGGAGAAAAAAACCTATGCAGAGACCCTACTACTTGTGG
15 TTTCTATTGTCCTATACATCATTCAAGCAAATCTACTGGCAGTTCTGTCAAGT
CCTCAGTGCATATGCTGCACAAACAAACAAACAAATCTGCATGGCACCAAAACC
AAACAAGCAAACCAAAACCCAGACACCCTATGTATCTGTTGGAGGCATGTAGGTG
GTACAAATGACTAGCCATGAGCACACATGGCTTGTCACTTCATAATT
TTTACTGCAAATGATTGAGAGGCTTGGTGCAGGCAGCCATTAGCCTGCTCCTT
20 GTTACCTCTGGATCACTTGCAGTAAATTGCAGGTCTTAAAAGATTCAAGCTCGG
TTTCTCAAAACAAACAAATTATCCTGTCTTACCTGAAAATGCAGGGTTGGGCAA
AAGAGGCTGGTTATAATAATGCCCTCATATTGAGTGGTGTAAATGGCTGCACACT
TCAGGCACTAGAGTTGCCAGGATGCGTTGTTAATGTGACCTGACTGGCTTACAG
GGGTGTAGAACAGTCTACACGGCGACTATTGCATCCATCTGCTCTCGAGGTGGA
25 TGGAAATAAGAAAAGGCTGGAGTGTGTAAAGTCATGCACATAAGTATTCACTGTAAA
TTTATTTCAACCAATTATGGTACTTGTCCAATGCACAACTGATCTCTCA
GTAGATATTCAATTGAAAATAGTGTGGCCTGACCAGCGAGAAGGGGAAGAAGTGA
CTTAGCTTGTGTTAAGATGACCTGTTGCTGAGAGTGGTCAATTCTGCAGCACCCCTAAAT
GTCATGGTTTGATTAGGGAGAGTTAATGTTTGACCTGAATTGAGTTCTTCTA

TTTTAGGAAGTATCAGAATTGCTCTGATGAGTAACAAAGTTGACTGTTTGATGTCC
AATCTCAGGTTTAAAATAGAGTGGTATAAAAGTCCACTGTTACTAATTCTAACAGAC
AATTTGATTAGTGTGCCCTAAAAGTCACGTGCATAATAAGGCCTGCTCAGAGGGC
AGGGCCTCCATCTGTTGCTCCTTCCATGTTGTACGCACTTCACTGAAAAGGTGTC
5 AAGTGACTTGCATTGTAGATTCCATTAAACCCCCAACATAGTTCTCAAAGATAAA
GCACTTTGAACATGAAATACATGGGTAATGTGTGATGTGGATCATGGTTCTCAG
GCCCTAGATAATCCACTCTGAGTATTGTTCTATGTAAGGAGAATAGAGGTCTCG
CTAATGTTCGAGTTGTATTCCCTGAATGGAATGCACTGCTAGTTCCAATGGATGGG
AGAGTAAACACTGCTGCATTACAATTGATACTGTTGCTTCCCTGAGCCTTAAGGT
10 AACTTTCTTCTGTCAACAAACAGCACTGAAGTTCTAGTAAGTGAATGAGATTATCT
GTTTCAGGGTTGGTTTAGAGTACTGTAAATTAAATTAGCTGTCTCCTAAAGAGGA
ACTCCCTTAACTCCCTCGATAGACTGAAAGTGGGTGTGGGGAGGGGGAGGGAAAG
AGAGGGAGGTAGTTGTAGAAAAAAAAAAAAAAA-3' (SEQ ID
NO:40).

15 Northern blot analysis using a sequence from the R055 clone revealed the presence of a
7.3 kb mRNA transcript. In addition, this analysis revealed that the expression of the R055
mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R061. The following nucleic acid
sequence is within the R061 clone: 5'-GGCCCCCCTANAAGGTCGAGGNTATCGATAAGC
20 TTNAATATCGAATTGGCACGAGGCCACCAGGTCTTGCATTGTCTTTAAAAGTG
GTGTATAAGGGGAAATTGGCAAGACAGACAGACATTCTAAACAGAGGGAACACAGAC
AGACAGACAGACAGACA
CA
TGTGGCATAACATACAGCTGCATGGGAAGCAGCCCCCTGCRCATTGCTTACATC
25 CTCGAGTCCTTCATCTTTCTAAACGTGTGCACCCGCTATAAAGTGGGTGATG
GGCTCGTCAGAGCTGGCTGATTCTGTGGCCGGTGACCACCATGCCTCAGGTCCCTC
AACCTCCATACCCATGGCCAATCCATAACTGCCACCCCTGAAAACCCAAAGCAGT
CTGAGGGTGCTCTGCCTGTCACTCAGAGGCCTGGACGTTGAACCCAAAAAGCT

AAACTTATGAAAGCCGGGCTGAAATGGGGCCCGGGCCTGGATAGCTCAGGCAGG
GGTTTCCACTCTGATGTTCCACTGGGCCAGTTTGTCTCTATTCTCT
GTTCATCCCGCTGAGTGTGATCCATGATGATTCCAGCATGAAGTACGTAGCACA
CTCCAGTTAGGAGAAATTAAAGATAACAAGACTAGCGTGGTGGTGGATGAGATGAGAT
5 AGTCTTCTCGTGCAGCACACCTGAAGGGCAATAAGGACAAAGAAGGCCATGT
GGCAGGGTAGCCCCCTCCAGACCAGGGTACAACGGACAGTTGTGGTGAGCCTCG
GAAAGGCAGGGTAACCTCCCTCCGTTCTCACCCATGCCAGAGCAAGGCAGG
TAGTCAAAGGGATATGCTGATGCAGAAAAGCCAGCTCAGGCATGGCAGGTGGGAT
TTATAGCTGGTTTGTAAAGCGAACGCCATGATATTGATAATGCAGTAACCAGC
10 GGTTGAGAGTGACAAGCCCTAAATGCGAACATTAATCAAAGGAGAACTAAACGG
CCCCCTTACAGAAGGACTT-3' (SEQ ID NO:41).

Northern blot analysis using a sequence from the R061 clone revealed the presence of a 4.9-5.0 kb mRNA transcript. In addition, this analysis revealed that the expression of the R061 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R066. The following nucleic acid sequence is within the R066 clone: 5'-CGAGTTTTTTTTATGTACTTGAAATATAT
TTAAAAACATTAAAAATTCTATATTAAAACATATATTATGTTAATTGGTACACTT
AAATAGAACCTGTATTACAATAGGCTCTGATGTGGTAAGTTAATGCCAATT
TTTTCAATAACATAATTATATAAAATATACTAAAATACAATAAATATTCTTGT
TACATGGGAATAATATCTTACCATAGAGAGAACAGGCCACAGACATTACTAC
AGTTCAATGGGAATCACTATAAAAGCATCAGGCCTGCTGCCATGCATGAAACACT
TCTGCCAAAAAGAGACCACAGCAAGACTTCAGAACAGAACAGAACAGGAC
GGAAACAGAACGAACAGAACAGAGGAGAGATTAAACAAATCAATCTCAGGTCAA
CATAAACCACCGACATGGAGCTATGATGTATCTTAGTGGTATGAGAGGCCAGCCACT
25 GACCACACAGTTGGAGGGCTCCTATGAAGCCACCTAATCGACCTGGCCCTCGA
ATACCGTGAGATTGTGATGGGCTCCTTATTGTTGACTAACGTCTCTCAGAATG
AAGCTGAAAAAGTTAGCATATAGCAGATATTCAAAGCATTCTTAATAGGTTAAA
ATGATGACAGAGATTAATGTTGCAAACGGCACAAAACAATCTAGGCTACGTGAAG

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TCTTCCAAAAACAGGGGATTCACTGGGACTCCAGAAGACAGACTAGTTCTAAAGGA
ACAGTTGAACAAAAAGAAACTATTGCTGATGGTATCTCACTCCCTGAGTCACAGT
GGACAGCCACTTGTTCACCCCTTCCACTCCTAACAGATGAAGCAATTGTTGCCTCTT
TTCTGATGCCAGGAGCCCAGTCAGGTAAACCACTAACACATTCGCGCTGGCGGAAA
ACCTCACTAGGGAAATGGGCTTAACACTAGTTCTCATTGGGCCATTCACTCAGGCT
TCCAGCTGACTTCTCCTAACCCCCAAGAGGTAAAGTGTAGAAGGGACCCTGTGCTG
AATGGACAGAACTATCAGGAGCTTCTGTGCTCTCACTAACAGCAGTATTCCCTCCTG
TGTCTTGCTCTTCACAGTGAAAGCACCTCCTATGCCTTGTCAATTCTAGCCCTTAC
AGACAGACATTGCTCATTGCCTAACAGTTGGTCTTTCTGGTTTGTGTTGTGTTGT
TTCTTCTTCTTTCTTCACCAAAATGTCTAAAAAAATAAAATAAAATAAAACC
TAGGCTCCTGAAGTCTAACGCAAAGAAAGTTAAGTCTTCACAGCAAACATTTC
CCATCATGCTGCACTGATAGCATCACTGCTATGCCATTGGATCCAAGCTGCTC
CAGGTTAACCAACTTATCCATAATTATTAAAATGGGATGGAGGCCATAATGGA
TTGAG-3' (SEQ ID NO:42). This clone is similar to BDNF.

Another IEG nucleic acid clone was designated R089. The first library screen produced a clone having an insert of 0.5 kb. A primary screen with a portion of this clone produced seven positive signals that were isolated. The following nucleic acid sequence is within the R089 clone: 5'-AGTCTGGGACTAAAACGTCACAGCAGAAAAAAAATAAAAAAAAT
AATTGCTTTCTTCATTAGCAGCATAATAAGTTGGCCACTGGGAGTAC
AGTACAGGGTGGACAACGATCCGTATTGAAGACCTACTTCTAGCACCAGC
AAGAACTAAATCCACCTCAGGACTCACAGAACCCAGGACAACCTGCCATTTGAGC
AACATATGCATTGAAGAGTGTATAGAACAGTAAATAGATTAACAGAGGCT
AATACTGTGATTGATTGACATTGCAATGGTGGCAAAAAAAAAAAAAAAA-
3' (SEQ ID NO:43). A portion of R089 was found to be highly homologous to a region within an EST from GenBank representing a cDNA clone from ae87b04.s1 Stratagene human schizo brain S11 (accession # AA774778).

Northern blot analysis using a sequence from the R089 clone revealed the presence of a 3.8 kb mRNA transcript. In addition, this analysis revealed that the expression of the R089 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R095. The first library screen produced 5 a clone having an insert of 2.0 kb. A primary screen with a portion of this clone produced 53 positive signals that were isolated. The following nucleic acid sequence is within the R095 clone: 5'-ACTTGATAAAATTGTATTTTTCTACAGTCATTGTACAATTG TTACAAAACCATAAGAAGACTACAACCTGTTAAATCATTGGTCTGCAAATATGT AAAATCTGTGGTGCAATTATCATGTATTACAGGGCCTGTTAGTCATTCAATGAT 10 TATTCAACAATGTCACACTCTAACATAAGACATGGCTTAAGACAAATATATTAGT ACATANATATTCTGAGAACATATTCCATNAATGGAAAGTNGCTGCTAACANATA CAGAATATACATAAGNTGTTCTAGCTTTAAAACAGTTTAAAATGGNAANGT GAAAAAAAGAGCCCCTAGGANCATTTATCCAAAAAAATCCTACNAAATATTNAA GGGGCCAGGGGGGAATTAAAACTAAAAANGTGGTC-3' (SEQ ID NO:44).

15 Northern blot analysis using a sequence from the R095 clone revealed the presence of two mRNA transcripts: one 2.5 kb and the other 3.2 kb. In addition, this analysis revealed that the expression of the R095 mRNA was extremely strongly upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R113. The following two nucleic acid sequences are within the R113 clone: 5'-AARGGGRCCACCCCACCGSGCTA 20 AAGGCCAGGGCCCCCTGGAGMCCCAGGGTTTGGCCMCCCCCTCACCC AAATGGTCTGCCAATGACCCAGGTACTCACACATGTTCCAGGAGGAGMCTGGGGC CAGGATTTGACCAGAGGGTATGGAAGGGAAAGGGAGAAGAAATCGACATTAT TTTTATTATTATTAAATGTTACAWTTCTTGTTCCAAGCCCTGAATAG 25 AACAGATAGCATTAAAGGACTCTGTTCCCACCCCTCTGTCTCTCTCCCCAC TTGTGCTAACTTAGGATAACACTCTCTATTGTTCTAAAGTGATTGTGGA CTTGTGCCGTGTGAAGTGCATTAAAAGGTTCTGTTCAAAGATCGATTGTCGTTCC TGTGGGGACAGTGGCTCTAAGAAATCTGCATTGTAGGAGAAGACAATGAAAGACC

CTGGCCCTGTCTCTCAAAACTTAACACTCTGTATGATTAAAAAAAATTCCATTAC
TTTACTTTGTGGTTACTTGATTGAGGAAGAAAATTCAACTTGTATAAAGACTA
GGTATCAGGGTTCTTGAGTGGAGTTGTATATATATCGTATTGGTATATCGT
AGAAACTCAAGCTTATGCATCCGTATTGGATATGTCAATGACGTGCAGTGAAAT
5 TTGCTATTAGACCCTGGAGGCCAACGAGTTGTACAAGGTTATGGCTCCATGGGA
ATTCTAATTCTTCTGGGGACCTTTGCCCCGTTTACAGTAATGGTAAATGGT
CCTAGGAGGGTCTCTAGTCGAAATTCTCCAGGCAGGACCACGTGCTAAAAATCT
TTGTATAGTTAAATTGGAGGAGTATCTCTGCTCAGAACGCATCTGTGGTGTG
TGTGCGTTGTTCTGTACTGTGTGACACAGCCTACAGTATTGCACTAAGGA
10 AAGCTGTTAGAGCTGCTGCTATGGAGGGAAAGAACATATTAAAACCTATTCCCT
CGGGGWTRTRWCWMGTTTATGTWCTTGTCTGGCTTCCACTTCCACT
GAGTAGCATTTGTAGAATAAAATGAATTAAGATCAGMWRWRWRMAAAAAAAA
AAA-3' (SEQ ID
NO:45) and 5'-AATTCCCCATGGAGCCATAAAACCTGTACAACACTCGTTGCCTC
15 CAGGGTCTAATAGCAAATTCACTGCACGTCAATTGACATATCCAAATACGGATGCA
TAAAGCTTGAGTTCTACGATATACCAAAATACGATATATACAACACTCCACTGCA
AAAGAAACCTGATACCTAGTCTTATACAAAGTTGAATATTCTCCTCAAAATC
AAGTAACCACAAAGTAAAGTAAATGGAATTTTTAAATCATAACAGAGAGTTAAGT
TTGAGAGACAGGGCCAGGGTCTTCATTGTCTCCTACAATGCAGATTCTTAGG
20 AGCCACTGTCCCCACAGGAACGACAATCGATCTTGAAAACAGAACCTTTAATGC
AGTCACACGGCACAGTCCACAAATCACTTNGAAACAAAACGAAATAGAGAGTG
TTATCCTAAGTNAGCACAGTGGGGNGAGNGAGACAGAGAAGGGTGGAACAG
AGTCCTTAAATGCNATCTGTTCTATTCAAGGCTTGAACACACAAAGAAATGTAAA
CATTAGNATAATAAGAATAATGTCGGTTCTCTCCCCTGCCCTCCATAC
25 CCNCTGGCAAAATCTGNCCAGGTCCCTCCGGAACATGGTNGAGTACCTGGTCCA
TTGNAGNCCATTGGNGAGGGCGTGGCAA-3' (SEQ ID NO:46).

Northern blot analysis using a sequence from the R113 clone revealed that the expression of the R113 mRNA was upregulated in response to the multiple MECS treatment. Specifically,

R113 mRNA expression was induced seven fold by the multiple MECS treatment as determined from Northern blot data using total RNA from rat hippocampus (Table I). In developmental studies, the expression level of R113 was found to be low and unchanged in embryonic as well as post natal development.

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TGCATGCACTGTGTTAGAGGCTGGATGTGACAATAATTGGGAGAGGCAGGAA
AGGAGTCCAGGACAAGCCTATGATATTCCCTCATTACCTTACCCAAGACCTCATTG
AACATTCTATATGCAAAGGGGCATTAGCCCTCAGGTTCCCAGAGGAACCTCCAAT
AAAGACCTGTCTCAGGGACCCCCAACCATTTTAATGGTCTGCTCCCTGACAAGG
5 CACTGATGCAGGCAAGGGGTTGTTTAAGGGTTGGTATCCCAGAATGGAG
CACCGGAAATAGGAAAATCCCTATTATAGCCCTCCTAGGACCAAGATTACCCCA
TGGCTGGGTGCTGGGACGCAGAACACAAGCAGAGGGGTGCGTGCCTGCGTG
CGTGCCTGCATGTGGTGTGAGGAAGCCTGAGATGCTCCAGATCTCTAAAGTGCAG
AGGAGAAGCAATGTGCCTCACCCCGTGATTCCATAAGCAGCCATCTGAGAGC
10 ACACTCGGCTGCCAGGAGGAAAAACAGGTCAAGGCCAATCTCATGGTTATCAATGGA
CCCTAGAGTCATACGCTGCCTGGCCAGCAGTGAGAGGCCATCCTGACTCCCTGTTG
CCTATCTTAATGCTCCTGCAGGGCAGCAGATGGTGGGGTAACCCAGAGATAATAC
CCATACATTGAGAACATTCTTAGTCTACATCTCATAGTCATTAGCGAACTGGACA
CATCTACCCGCATCACCCCTGGAGGTCAACAGGGACCCCTGAGGGTGGGCTGATGC
15 CAGGCACTTATATAGTGAGCAGGCGTGCAAGTCTGGGACCCAGGGAATCCATCTCA
GCCCCCACCCCTAGCCAGGAGAGAACAAAGTAGGCCCTGTTCAAGCCCAGCTCG
GAGGCTGCCTAGCTCCTCCTGCCCTCCTGCAGACCCAGCTCAGCTTGATGAG
GTGTGACAACGTCAATTAGAGGAAGCCCTGCTGCCCTGAGCATTAGAGCA
AATTAGAGAAGAAAAATCACAGAGAACGCTTCTGCCTGAGTCTAGACTCCCAG
20 GGGACTGGGTGGAGGAAGGAAGAGCTTAGGGCATAGGGATGAGGAGGTAAAAGTA
ACAGCAGGAAGGGTCACCTGCAAGTCCCACGCAGTAAATGATAGGTGGCCTTTT
TTTTTTTTTAATCTGTAGCTTTGTCAGGCAATGTGCCTATCTCTTCAGAACAAAT
TAATCAGTGGGTCAAAGGCCCTGCCATGCTGGCTGCCCTCAGGCTACTCAAA
AAGGAAAGCAGTCCAAGCTCCAGCCTGAGGCATCAGGCCTACTGCTCTGGCCTG
25 GTGTTATCAGCTAGGCTCGCTTTCTGGTCAAATGGTCCTCATCCATTCTGTCCC
CACTGAACCTCTGTCTGGTGAAGGAAGGTAAGTGTAGCTGCCTGATGGCTGCT
GCAATGTGTGGAGAATGAACATGTAAAACCCACACCCCTGAAGGGTGGCACAT
ATGACACATTTACTCAAGAGGACACAGGACTGGGACGGTAGGAAGCCAACTCAT

TTGTTTGTGGACTAGTCACTGTCACATTATTAAATCGACTGACGTGACAGACTCC
TTCTTGACTGGCACTGTGACAGAAGGAGAGAACTCAGCAATGGAAAGCTGGCC
TCCACAGCTACCAAGGCACACAAAGAAATCCAGTTAACCAACCACCTGGCCAGAAAA
GGGTCAAGGGACCAAAACAAAATGATTAGCAAGTAATTGGCTCTAAGAGAAC
5 CACAGGTGTCTGTCACCTGATCTTATTTCTGCTACACCCAGGAAATGGTTGCTC
ATTTCACCCAGTAGACTCGGAGAAGTTAATGCTTCAAGGTCACACAGTACAAAGCT
GGGATTGAAACAGTTGTAAGTCAACTGACTCCAATCTGTGTTCATGCTACCTGGCAAAC
TGTCCATATTGCTCCACAGCCAGATCCAGAATAAACATTGTCTCCTCTCGTGCAAAA
AAAAAAAAAAAAAAA-3' (SEQ ID NO:47). In addition, the R114 clone contains an ORF
10 from basepair 94 through basepair 993. This ORF encodes a polypeptide of 300 amino acid
residues. The translational start site was assigned to the first methionine residue in the ORF.
The amino acid sequence of the R114 polypeptide is as follows: MKKESRDMDCYLRRLK
15 QELMSMKEVGDGLQDMNCMMGALQELKLLQVQTALEQLEISGGAPTFSCPSSQEQT
ECPRWQGSGGPAGLAACPSSQPSFDGSPKFPCCRSCIGKELAVLPKTQMPEDQSCTQQG
IEWVEPDDWTSTLMSRGRNRQPLVLGDNVFADLVGNWLDLPELEKGERGETGGSGEP
20 KGEKGQSRELGRKFALTANIFRKFLRSVRPDRDRLLEKPGWMTPMVSESAGRSKKV
KKRSLSKSGSRFPFSSTGEPRHIETPATSSPKALEPSCRGFDINTAVWW (SEQ ID NO:48).
A portion of R114 from base position 111 to position 210 was found to have 98 percent identity
with the mouse G protein-coupled receptor EBI 1 (accession #L31580). This homology,
however, ends with position 210. In addition, the 100 bp region of 98 percent identity in the EBI
25 1 clone appears to be an artifact produced while PCR cloning EBI 1. This “identity” region in
R114, however, is not an artifact, since RT-PCR with primers located in the 3' untranslated
region of R114 and the middle of the “identity” region (139-164 bp) was used to obtain portions
of the R114 clone. In addition, a portion of R114 from base position 143 to 601 was found to
have very strong homology with a human EST obtained from prostate tumor (accession #
AA595469). This indicates that the entire “identity” region is from one gene and not a product
of concatamerization of the R114 clone and EPI 1.

The alignment of the human EST obtained from prostate tumor with R114 revealed a

very high level of identity at the 5' and 3' ends of the overlapping region and a somewhat lower homology in the middle. In addition, 13 base insertions and deletions were identified between the EST sequence and R114. After excluding 7 of the 13 differences because they would have caused a frame shift, the two sequences were translated and compared. This comparison
5 revealed an 81% homology at the nucleic acid level and an 85% homology at the amino acid level. Interestingly, no homology was found between the two sequences before position 143 of R114. Position 143 is six bp before the third methionine residue. Thus, the translational start site of R114 may be the third methionine residue in the ORF.

Further, 95% homology was found to exist at the nucleic acid level (98% at the amino acid level; there is a one base deletion in the EST that is probably an error of sequencing)
10 between the 3' end of the R114 ORF from position 580 to about 987 and the full length of an EST from mouse mammary gland (accession # AA472513).

Northern blot analysis using a sequence from the R114 clone revealed that the expression of the R114 mRNA was moderately upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R198. The following two nucleic acid sequences are within the R198 clone: 5'-TTTKTTKTAATTTTTTTTNTATTGGGTTGA
15 TTCCTTGTTTANTGCCAAATNTTACCGATCANTGANCAAAGCAAGCACAGCCA
AAATCGGACCTCACCTAATTCCGTCTCACACAAAAATAAAAAACGGCAAACCTCA
CCCCATTTTAATTGTTTAATTACTTACTTATTATTATTATTGTC
AAAAAAATCTCAGGAATGGCCCTGGGCCACCTACTATATTAATCATTGATAACAT
GAAAAATGATGGGCTCCTCTAATGAAAAASCAAGGAAAGGAAAAGGCCAGGGGA
ATGAGCTAAAATTGATGCCACKTGGGAGCATCTGGTGAATAATCGCTCACKTCT
20 TTCTTCCACAGTACCTGTTGATCATTCCACAGCACATTCTCCTCCARAAACSC
GAAAAACACAASCAGTKTGGGTTCTGCATTAAAGGATAARARARARAAGAGGTTG
GGTATAGTAGGACAGGTTGTCAGAAGAGATGCTGCTATGGTCACGAGGGGCCGGTT
25 TCACCTGCTATTGTTGTCGCCTCCTCAGTTCCACTGCCTTATGTCCCCTCTCTC
TTGTTTAGCTGTTACACATACAGTAATACCTGAATATCCAACGGTATAGTCACAA
GGGGGTAATCAATGTTAAATCTAAAATAGAATTAAAAAGATTGACATA

AAAGAGCCTGATTTAAAAAAAAAGAGAGAGATGTAATTAAAAAGTTATTAT
 AAATTAAATTCAGCAGAAAAATTGCTACAAAGTATAGAGAAGTATAAAATAAAAGTT
 ATYHGTTCAAAMTAVCDCRTCGAMCTCVTCVABCCCGRGGAAKCCMCTASKKCBA
 RHSCGGCCCCACCSCSSYSKAKMTYCATAKCTTGAWWWCCCTTAGTGAGGGTTAA
 NAA-3' (SEQ ID NO:49) and 5'-CAGCCTCTCACTCTCTNGCTCTCTGTCTCCCT
 CGCTCCCTCTCTTCTCTCCCTGCCTCCCAGTCATAAAAGTCTGTGCGCTCC
 CGGAACCTGTTGGCAATGCCTATTTTCACTTCCCCCGCGTCTCTAAACTAACTA
 TTTAAAGGTCTCGGGTCGCAAATGGTTGACTAAACGTAGGATGGGACTTAAGTTGA
 ACGGCAGATATTTCACTGATCCTCGCGGTGCAAATAGCTTACCTGGTGCAGGCCG
 TGAGAGCAGCAGGCAAGTGCATGCAGTCTTAAGGGCTTCAGACTGTTGCTCA
 AGCTGGGTGACAGCATGCCAACTACCCGCAGGGCCTGGACGACAAGACGAACATC
 AAGACCGTGTGCACATACTGGGAGGATTCCACAGCTGCACGGTCACAGCTCTTACG
 GATTGCCAGGAAGGGCGAAAGATATGTGGATAAACTGAGAAAAGAACATCGAAAA
 ACCTCAATATCCAAGGCAGCTTATCGAACTCTCGGGCAGCGGCAACGGGGCGCG
 GGGTCCCTGCTCCGGCGTTCCGTGCTCCTGGTGTCTCTCGGCAGCTTAGCGA
 CCTGGCTTCCTCTGAGCACGGGCCGGTCCCCCTCCGCTACCCACCCACACTC
 ACTCCATGCTCCGGAAAATCGAGAGGAAAGAGCCATTGTTCTAAGGACGTTGT
 TGATTCTCTGTTGATATTGAAAACACTCATATGGGGATTGTTGGNAAATCCTGTTTC
 TCTC-3' (SEQ ID NO:50). This clone is similar to neuretin (accession # U88958).

Another IEG nucleic acid clone was designated R233. The following nucleic acid sequence is within the R233 clone: 5'-AAACCNAGAACCCCCCTTGNAGAACCCNTG
 TTCCCTTCAAGCCAAGGAAGGCGGGGCCAACCTTGGTGTNTTGAAACAGGCC
 TTGAACAGGAGGNTWAGGAGAAATTCCGGTGTGGAACCCCAACAGGAACCCCTT
 GGCACCCCTGGCCCCAAGGTTGTGMAACTTGGTTGCTTAATTGGACCGTTTG
 CTTGAGGATTCATGACTTTTGTGKGCCCTGTGAGCCAAGATGTTGGTTTCCA
 TCAACAWTAATAACCCCTGCTTTGGGTGATTCCCTGGGAGTTCTGATGA
 ATTCCCCCACAGCTCCTGGGTTTCATCTGTTACTGTTGTCTGGATTAGGAGG
 GCGGAGAGGGTGGACTCCCTGAGACAAGATAAGCAGGTGGAGACATAGAAGAGGG

AGGGACATTAACATAGAACATTTCAGAGGTGACAGAGATGATA
5 CACACGGGCAGCTGGCAGACCCACAGGGCCATA
TGGAMTTTGTGAAGGACAGAGGAGCTGGCAGACCCACAGGGCCATA
GACAGGTGAATGGCTGGTTACCAGAGACAGGACTGGTAGACAGTCAAGTACCTCAC
TACGATGTGCCAAGAGATYTGGATCCTGGAAATGTGTGGAGAAGAGGATTGAC
ACTCCCCACCCCCAAGGCCCTTCCCCTTGCTGACAGCATTGCTGTGGCGTGGCCTG
TTGCCTTGTCCCTGTCCCTGGGTGGGGCACACCCCTCTGTGCTGTGCTTGCCTGTG
CATCAATAAACAC-3' (SEQ ID NO:51). This clone is similar to KIAA0273 (accession #
D87463).

Another IEG nucleic acid clone was designated R241. The first library screen produced
10 a clone designated R241-4. This R241-4 clone contained a 2.0 kb fragment and a polyA tail. A
second library screen using 5'-end of R241-4 as a probe produced an additional clone designated
R241-12. The following nucleic acid sequence is within the R241 clone: 5'-GCANTTTGGAGT
15 TATTGCTAAAACCAGGNTAAGGCACTTGTCCCACAGGACCCAGGAATCNTAAAN
GGGTTGAAATTGGNCGGGAACCCCAGGATATAATGCNACTTTGTTAGGGGGAG
AGTTCAGCTCTAACTGGTAGTAGTGTGAAAGTAAGCACCTGACTCAATTGGAA
AGCACTTGGTAAATGGAGAGAACCTTGGAGTTCCATCATCTATATCAGTCTTG
AACACACCCCTCAAGTCCCAGCCTCAAGGCTCAATAAAGGACCACATGCAGGTCTG
AGGCTCACTGCTCTCAGCCCTAACACAGGGCAGTGGAGAGCAGGGTGTCTCCCT
20 CTCTGGAGCTTCTCCTGGCCTTCTCCACTTGGGCTTCTGCTCAGCAGCAGATAT
ATTCTGGGTTCCATAAGGAATCCAGCTGCTCCAGTGGCTTGACCCGTCAAGGCAAG
ATATCAAACCTGAGGATGACCCAGTCATGGAGGAAGAGAGTGTGACAAGATCCGCA
GTTTGAAGCAAAACTGTGTTGGCTTTCAAGAAACAAATGGGCACATTGAGTTCT
25 GTTCAGTGTCAAGAGGATATCTTCCCTTGCTCCCAGATTCCAGAAATGGATAATGT
TTTCATTCTGTGGGAAGGGTCAAGAACATAAAATTGCTCAACAATGCTGCTTCC
CTTGAGGGTTGTTGAGCAAAGGCCGATATGCCTCCCTGCATTCTCTTACCTCAAG
ATTGGAAATTCAATTCTGGAACAGAAATTATACACAAGAACACTTGTGTCAG
CCTTGGTTACTGTGGAGTTACATAAGGGTGACAGTCTGTATCTTCTAARTAAACA
GGAACGTGGCTTGGCGGCATTGACCCAGTTATCTAAATATAACTGTGGCTC

20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3

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CAAATGATTGGCCAATAACATTCCCTTACCTTCAAAGTTCTCCATCAGTCATTCT
TGTGGCAGCACAGTCCAATGTCATATGCC: TGCAAATTGTGAAAGTAATTAGTGA
CAAAATAACCCTCCCCCTTCAGTGGCCAAACTGTCAGCTGTAGCAGCGCTGCGAA
AGCGAGTACTACACTATGTACGGAAAG:CCTGTTCCCTATCACGGACTAGACTCAAG
AAATGCCATCTCCGAACGGTGGCATTCAAGGTGGTAGTCGTTGAATGGAACAGTC
TCTATGTGGACATTGTTAAAGTGTAAAGAGTATTGAAAATTAAAGTTACATT
TACAACGTCTTATTGAAACAATTGTATATAAATATTACCCCTTCACTGTT
AATTAAAGTAAACCTAGACCTTGTAGACAAGTGGTCAACTGATATGTATAGAACAGCT
GTGATGTAGACAATACCTTCTTGTAAATGGTCATAAAATATAGCTGTTCTGTG
TTTTATAAGTTGAGGGTATTTGTTGTTATAACAAACAAAATTATTGCATTGAA
ATGGTTTTATGTAATAGAACATGCAAACAGTGAAGGATTATAACATGGTATATGT
AAATGTATAAACTTAGAAAGAAATAACACAAATTCAAAAAAAAAAAAAAAA
AAA-3' (SEQ ID NO:52).

Northern blot analysis using the 3'-end the R241 clone as a probe revealed the presence of two mRNA transcripts: one about 7.0-8.0 kb and the other 4.8 kb. In addition, this analysis revealed that the expression of the R241 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R256. The first library screen produced a clone designated R256-8. This R256-8 clone contained a 1.8 kb fragment. A second library screen using 5'-end of R256-8 as a probe produced two additional clone designated R256-2 and R256-3. These additional clones contained each contained a 3.0 kb fragment. The following nucleic acid sequence is within the R256 clone: 5'-GGCACGAGGACAGATTCTGAGA
TGGAAACTTAAATTACATCCCAGAGGCAGGGAAACTATGAAGTCACCGTTCTAGA
CCACCCCTTACTGAGGTTCCACGGTCACACTGACGGCAGGACCCACAAGGGCAGGG
TATTGGTCTGCCCTCCTTCTCCTGTCTGACTTACCTAACTTGGTCTCGGCTGC
TGACACTTGGAAAGGACCAAATTACTGATAGTATTCCCCCTGTTGTAAATAGC
CTGAAACCTTGGAGAGGTTCCAGAATACTTCTGTATATAAGGGCACAGGTGAAGACAT
TGTCCAAAGCTATTATCTATTACCCCTGGCTGAGTAACCACACCAGTA

GGGGGAAA ACTAAA ATGTGTTGAGTGTAAACAAAGTCACCAGCCTGGCTAGAAATT
CTCCCTGGAAA ACATCCATTTGATA CAATGTAAACGTTAGTGTTCACCCTTAGATA
CATGTTGAAAGAGAGCTTGGTACCGCGGAAGTGGCATCTTGGTCACACACCATGCC
AAAGTGAAGAGGGTGGCCAGTGGAGGTCTCCGGCCTGTCGGGATCATTGTGAATA
5 CATTCTTGCCCCTTAAGTACTTGTACTAACATGTGCAGTGGTAGGTATTAGT
GTTAGATCACAGTGGCACTCCCTGGGATCTGGGAAGACCAAGAGCTTGCAACTC
TGCCTGTTGATCCCTATTCTCACAGTGTGTATTAAAAAAAATAGGATTAAGAC
AGATAACCACCTTACATTGTGAGTGTGTTGCCTGTCTAACGACAGATAATTCCCT
AACATTCTCTCACCTTAGTACTTCTAGGCTAATTATACACGTCTGTATGCCATGA
10 GTAAGTGGACTGTAGTCGGACAAAAGAAAACAAATGAGCCGTTGGACCATTGTG
CAGTCAGTTCTGGCCTTAGATGTATCCTAACAGCAGTAAGTGTCTGATTGTACCCCTGG
TGGTATGATCAGTTGTCTCGTAGCTGTCTCAGCTCCACAGTTACAATGCAAATCTGT
CTCAAGATCTTCACGTCACTGCTGAGAGCAGGGAGAATTCTCTGCAGCTTTTC
15 AAAGTTGTGGCCCGGCCTGAATCCTCTGTTAATTACTGTGTGAGGCCAGAGGGAGCT
GCCCAAGCAAGGGTGGGCCAGCCGGCAGGGAACTTCTAGACTCCCCGCTCATT
CAATTGATCTAGGCATTGGCCTGCTACTTGACCATTCTGCCCTGTGAAATGTCCC
ACACTTGAAGCAAATACAATTCACAGCACAGTACACACACAAAAACCCCTGGCATAAG
20 ACAGGGGAGGTTCTTATTGTGAGCCGTTGCCCTGGAAACGGATAACAAAGG
GCAGCCTTCCACTCTGGCATAATGGTGGAGCCTCTTCAGGCTTGACACCTGTC
TGAATAAGAGTGATTAGAGCCGCATAATATCCCTCTGGCTATTGAATATGTGGT
TCACATACCAAACCCCTGTAGAAGTTAGAAGACGGTCGTGTTGTATGTTGCTT
25 CCACTACATTGAGGTTGTAAAAGTGTATTTCACGATGTGAAACTGAA
GGTCAATAAATTATTAGAGATTTCAAAAAAAAAAAAAAAA-3' (SEQ ID
NO:53).

Northern blot analysis using a sequence from the R256 clone as a probe revealed the presence of a 4.0-4.8 kb mRNA transcript. In addition, this analysis revealed that the expression of the R256 mRNA was moderately upregulated in response to the multiple MECS treatment.

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Another IEG nucleic acid clone was designated R261. The first library screen produced a clone containing a 1.0 kb fragment with a polyA signal and tail. A second library screen using a portion of this clone as a probe produced 41 positive signals that were isolated. In addition, PCR using T3 or T7 primers along with a R261 sequence specific primer resulted in the 850 bp
of additional sequence from a solution containing the phage plug from a first screen. The
following nucleic acid sequence is within the R261 clone: 5'-

CTTAAAACCCCTAGATTCCCTGTTA
CATACTAACACAGGTCTCCCTTCACTCCAACCCCAGGTTCAGGCCTCAGAGCCA
TGCTGGGGTTGGAGAAAAGTGCATTCTATGAGGGTAAAAAGTAGCTGCCCTCTCTG
10 ACCCTTCTTGCTAGGCTTCATGCCGGATGGGAGAGGGTATCCCCAGGATGGGGACA
GAGGAAGCCTGGCTAGGGCCTCTAGCCAATAAGCAAACAGGAACATAAGCAG
ATCAAAATCCTACACTAGCTTATTAGGGCCCTGTTAGTTGAAAACCTTGTGCTGTCC
CAAGTTCTCAGTTACAACCGAGTACACTTACTCTCCAACGTCCCTAAGGGTCACTA
CCCAGCCAGCTTGGATCTCAGCACCTTAAAAGCTGAAACTCCCTTGTGCCCTCT
15 TGTCTATTCTCACTGCCAGTTGGGCCTAGGCTCAGTCCTGGCAAATGCCATGA
TCCTGCTGCTGTGGAAAGTTGATAGGGCATTGGCTCAAATTCAAAAGGCCTCGC
TCCTGACCTGATTCTCGAAGCTCCAGTAGTTCTAGACCCCTCCAATCTCATCTGA
CTGGTTGCAAGGCTTATTTCTTGTACTTCCTATAGAGCATTCTGTAGCATTG
20 AGTGTGGCGATATTTGTTGTGTAGATTCTAAGAACCAACACTACTCAGTCTCC
TGCTAGTCTGACTCCTGAAGCATCAGACCTCGTCATACGGTATTGACTGTATGTG
CCTTCACCTTGAGCATGCTCAGGATTTCTAAACCACAGAACCTGAATACA
CAAGGGAACCAGAATTACAAAGTCCTATGCAACCCCTAGACAGGAGGAGGTTAGAG
AGTCTGTCTGATTGGTATTTCAGAGACCCNAGAGAAATTGTACCAGTTGTATT
25 AATGTCAGTACTACCAGCACTTGCCTAAAGGATGTCAGAGGGACCTGTTCT
AGAGTGAGTCCCAATTACATCAAAGGGCAACTTACAGCTTCTCCAGTAAGTCTGAG
TGGTTCTCTGAGCTGGTGTCACTTCTAACCTTGCAGTCTAGCCCAGCAGGGCCC
TGTGTGTGAGTGCAGTTGGTGTGTTGGAGTATGCCCTGCTCCCCAGCCTGGAA
CCCTCTCAGCAACTTGCTGGACCTATAATGTCTAGGTGCAACAAGGACCTACCA

GAGCTCCTGGGTGGCTTCAAGATCCACGTAGCTTGTGAGGGGACTGAATGCAG
ACAAACCACAGCCTGCTCAAATACCTCTTCCCTACCACCTAGTTCCAATGGAA
CCAACAAGTTGAGTCATCTCTGTTGGGTGTTGAGACTGGCTGAAGTGAA
AACTCTTGACTGACCATGTTGATGTGTCGACAGACTCAAGGACACAACCACCTC
5 GAGCTGGTCATGTGGCATGCCTGTGTATGTGTAACAGGATTCTGAATGTTAGGTT
GTAATGCTATTCCCTGTATGGGAGAAAAAAAATAATATAAACAAATAAATCTATTAA
AAGCACAAAAAAAAA-3' (SEQ ID NO:54). Sequence analysis revealed the presence of
some homology with EST sequences including that of a cDNA clone from ae69b04.s1
Stratagene schizo brain S11 (accession # AA774320).

10 Northern blot analysis using a sequence from the R261 clone as a probe revealed the
presence of a 4.0 kb mRNA transcript. In addition, this analysis revealed that the expression of
the R261 mRNA was marginally upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R272. The first library screen produced
a clone that was used in a second library screen. This second library screen produced two
15 additional clones designated R272-1 and R272-2. Clone R272-1 contained a 2.0 kb fragment
while clone R272-2 contained a 1.7 kb fragment. The following two nucleic acid sequences are
within the R272 clone: 5'-CCATGGGACTGGTTGTACCNATTGCCATGGNTTGGTT
GGTAGGTGTTTTGGACATTTCAGTTNCGTTGAACCTCCAGATTATTGGGT
TTTGTTTAATTATTTGTCAGAGGAAAAATAATTAACATCCATCTCACAGGCT
20 TGCTTGACTGTTCAAGGTCTGCTCACTTTCTTGTCTGCCTCTGCTCTGG
CTTCTTCATGATAGTGCTGGACGTGGAGCTGAGAGTCTCGTTACTCTAGGCAAAC
CCTCTACCTGAAGCCAGAGCCCAGCACTCCGTACCACCAAGACTCTGAAGCTGGC
AAAGTTTAGAAGCTGGAGTTCTGATTCTCTCATTATTAAGTTCTCCTCAGTCT
25 TTAGATAGAGGTAAATGTGGGCTTGTAAAGAAAAGAAAGCAGTAATGTACA
CCTATTCTGAATTATGCAAATTAGCTCTTACTCAGGGTCAACTAAATTACTCAACTC
GCCCTTAGTTACTCTTAATTGCAAAAGAGAAAAAGAAGGAAACTAAATAG
GACTATGATTGGGGAGCAAATTGATAATCTGATGTAAAAGTTGCTGTGTTAACAC
TAAATTATTAAGTGTAGACTTTCTAGGATATTGTATTGATTTGTGATATGCC

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TAGAATGATGTATTAGATAAAAATCAATTGTAAAGTATGTAAATATGTCATAAATA
AATACTTTGACTTATTCTCAAAAAAAAAAAAAAAA-3' (SEQ ID NO:55) and
5'-GATTTATATTCAATGTTGTTATTAAATCCATTGCAGTTGGTGAATGCCTTT
CCTCCTAGACACCCTGTATTATACCATTGGGGATTAAGTCAAAGTTAAGTATATT
5 TTTCTTACTTGAGCTCTATATGCAATTCAAGATATCTTCCTGATGACAGTTTATAT
GTAAATGTAATTAACTTCTTCCGTGTTGACGAAGTTCTGTAGGTGTTAGGGTTAG
AAGTCTCAGCACTCACTCTCTACTGGATGTGCAGTGTGCCATGGCGCACGG
CTTCTCAGTAATGATGCCATCTCTGCTACTTTACAGAAGGAGAAGTTACTTTGAG
GTGGGTATGTGTTGATATCTAACACTGTGTGTTGCTTAGATAGGCAAGACAC
10 ACTGCTGTGCGTGGCTCCTGTGGTCACCTAGCCCAGGGAACGTAGCCTCAGTACT
TCCGCTGGCTTCTTCATGCCTAAGAACAGCAGGGCCTTCTGTTGCTGGCTCTGGC
TTAAAAAGTTGTCCTTGGGTCTGGAGATGTAGCTCTGTGACAGAACACCAAGCTAAT
GTCAGGTCCCTCGGGTCAGTCTCTGGTACACACAAGCGCACACTCACATGATGGGGGG
ATGAAAGGCTGTCCTTGTAAACAGTATTGATGGGGCGTGCCTGGATGACGATGT
TTATGTACTCTGAAGGCAGATCCTGAAGGCACCCCTGTTCCCTTCCTGTGTAAC
GAGTCTGCACTAGCTAGCCACTGTTAGAGGCCATCCTAGTGGCGAACAGGAGG
CATCGCACTGGGTGATGGTTGCCTTCAGTCCTCAAGTAACAGCGGCCACTATGC
CGATGGCTTGGTAAATCAAATTACCAAGTTGGCCTAGTCTGCCTCTGTGAAG
AAGGGGAGAAAGGAAGGGTGGAAAGGTGGATGGAAAGCCTTGGGAAACTAGTCT
20 GATCTCTCAAGGG-3' (SEQ ID NO:56).

25 Northern blot analysis using a sequence from the R272 clone as a probe revealed the presence of a 1.0 kb mRNA transcript. There appears to be a discrepancy in the length of the R272 mRNA since the Northern blot data indicates a message of 1.0 kb while the cloning data reveals a message length around 2.0 kb. Regardless, the Northern blot data indicated that the R272 mRNA expression level was moderately upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R280. The following nucleic acid sequence is within the R280 clone: 5'-CTTCAGTTCCCTTGAGGGNCTTCCTTC

GAAGGGGATACGCCAACCTTCACGAGTTGCGCAGTTGTCTGCAAGACTCTATGAG
AAGCAGATAAGCGATAAGTTGCTAACATCTCTGGGCATAAGTCGGACACCATG
GCATCACAGTATCGTATGACAGAGGCAGGGAGTGGACAAAATTGAAATCAAATA
ATGATTTATTTGACTGATAGTGACCTGTTGCAACAAATTGATAAGCAATGCT
5 TTTTATAATGCCAACTTAGTATAAAAAAGCTGAACGAGAAACGTAATGATATAA
ATATCAATATATTAAATTAGATTTGCATAAAAACAGACTACATAATACTGTAAA
ACAACATATGCAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTAAC
AGAGCATTAGCGCAAGGTGATTTGTCTTGCCTAATTTTGTATCAAACCT
GTCGCACTCCAGAGAAGCACAAAGCCTCGCAATCCAGTGCAAAGCTTGATGCC
10 CAGGTCGACTCATATGCCGTGAAATACCGCACAGATGCGTAAGGAGAAAATACC
GCATCAGGCCGCATGCCCTGATAGACGGTTTCGCCCTTGACGTTGGAGTCCA
CGTTCTTAATAGTGGACTCTTGTCCAAACTGGAACAAACACTCAACCTATCTCG
CTATTCTTGATTATAAGGGATTGCGATTCCGGCTATTGGTAAAAATGAG
CTGATTTAACAAAATTACCGAATTAAACAAAATTACGCTTACAATTGC
15 CATTGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCCCTTCTG
CTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCATTAGTTGGTAAC
GCCAGGGTTTCCCAGTCACGACGTTGAAACGACGCCAGTGAATTGAAATACGA
CTCACTATAGGGCGAATTGGGTACCGGGCCCCCTCGAGGTCGACGGTATCGATAA
GCTTGATATCGAATTGGCACGCCAGCGATATGCACTCCCCGGCGGTGCTCG
20 TCACCTCCAGGCAAGTCAGAATGCGCACACGGYCTGACCTGACTGTACCACAGC
ACCAGGAGGTGCCGGTAAGATGATGTCAGGCCATGTGGAGTACAGATCCTGGTG
GTGACCCGGTTGGCTGTCAAGTCAGCCAAGCACCGGCCAGGATGTCGTCCAG
TTCTGGTCTCCAAAAAATACAGCGAGATCGAGGAGTTACCAGAAACTGTACAGT
CGTTACCCAGAACGCCAGCCTGCCACTGCCTAGGAAGGTCTGTTGCGGGAG
25 TCTGACATCCGGAAAGGAGAGCCATGTTGATGAGATTCTACGCTGTCTCCAAG
GATGCCAGTTGGCGGGCAGCCCAGAGCTGCTAGAATTCTAGGCACCAGGTCCCCG
GGGGCTACAGGCTTGCACCCGAGATCCCTCTGTTGGATGACGACAGCCAGCC
GGCCAGGGGACAGTGATGAGGTTGACTTGTAGCAACAGGGATGAAGTGCA

AGCCACCCACATTGGGCCTGAGCAACAANGAAATGTTGAGAAGGTCCNTGGAAGGA
ANGAGGAGGGAAAGGGAGGAAGGANGATAACTTGGGATCCCCCTGGGGCAATCAAT
GCGGCCTCCCAAAGGAAAGNCCTAAAG-3' (SEQ ID NO:57).

Northern blot analysis using a sequence from the R280 clone revealed that the expression
5 of the R280 mRNA was upregulated in response to the multiple MECS treatment.

Another IEG nucleic acid clone was designated R286. The first library screen produced
a clone that was used as a probe for a second library screening. Briefly, the ³²P-labeled probe
was used to screen a UniZAP rat hippocampal oligo(dT) primed library (Stratagene). This
second screening produced a clone having a 4.7 kb full-length R286 cDNA sequence. The
10 nucleic acid sequence of this rat version of R286 is as follows: 5'-

CTGCCAGCCGAGGCTCCTGCCGC

TGTGACCCCGCGCTCCGCCCGCCGGGGGGACCCCTGATAGCTAATGTCAGAAC
AAAGTGACTCTGTGAGAACCAAGCCCCTGTGGCCTCACTCTCCGAAAATGAGCTGC
CACCGCCTCCCCCGAACCTCCRGCTACGTGTGCTCGCTGACAGAACAGACTTGGTCA
CCAAGGCCAGGGAAAGAGCTTCAGGAGAACGCCCAGTGGAGACTCCGGGATGTGCAG
GCCCTTCGAGACATGGTACCGAAGGAGTACCCATACCTGAGTACATCGCTGGATGAT
GCCTTCCTGTTGCGCTTCTGAGGGCCCAGGGTTGATTATGACCGGGCCCTGCAG
CTGCTGGTCAACTACCATGGCTGCAGGCAGGAGCTGGCCAGAGGTCTTCAGAACCTG
AGGCCATCAGCCCTGAAAGACGTTCTTAACCTCTGGATTCCCTCACAGTGTGCCCCAC
ACAGACCCCAGGGCTGCCATGTCCTCTGCATCCGACCAGACAGATGGATACCGAG
CAACTACCCGATACCGAGAACATCCGCCATCTACTGACGTTAGAAAAACTCAT
TCAGTCCGAGGAGACCCAGGTGAACGGGGTTGTAATCCTCGCCGACTACAAGGGAG
TGAGCTTATCAAAGGCGTCTCACTTGGCCCTTATGCCAGAAAGGTGATTGGCA
TCCTTCAGGATGGCTCCCCATTGGATAAAAGCAGTTCACATAGTAAACGAACCTC
25 GGATATTAAAGGGATTTCGCCATCATAAAACCATTCTGAAGGAGAAAATTGCAA
ACAGGTTCTCCTCCATGGGTCTGACCTGAGCTCTGCACACGAGCCTCCAAGGA
ATATCCTCCCCAAAGAGTATGGGGGCACCGCTGGGAGCTGGACACTGCCAGCTGG
AACGCGGTGCTGGCCTCGGAGGATGATTGTGAAAGAGTTCTGCCAGCCTGAG

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TCTGGCTGCGATGGTCTCTGGGCCAGCCCTGCTGCCTGAGGGCTGATCTCAGAC
GCGCAGTGTGACGACTCCATGCGAGCCATGAAGTCCCAGCTACTCCTGCTATTAG
CCCTCTCCGGAGAATCACCATGTGTAATTCCCTCCTTCGAATGCACAGGCTGA
AGATGCCAGGACCTCGGTCTGCTCCATCACAGTGCAGCACGGAGCTGCCTGCAGAG
ATTAAGGAGAGCCCATCACAGGCAGACCTCTGACCAGCTAGGTTATTCCAAGAAG
ACATGGAAATTGCCCTGGTGATTCCCAGATGTCTGTACTCTAAGTCTGCAACTGTTA
CTCTGGAAGCTGCATCTGTTCTATGCATCTGGAAAGAACTAGGGTCAAAGTCAC
TCTGAAGTGACCAGGAGTAGACAACCTGATTGATCATGAGTCTGAAACAATTGCCAA
TCCTGAAAGGTGCCATGCGTGAGACTTGAGTCTTCCCATAAAACTGTAGGTGT
TGACTACTGCTGTTATCTGCAAAGGTCAAGGTTAGGGCTCAGGCCAGTTGGCATTGCTGG
GTCTGGGAAGCACTGCTAACTGAGTGGTAGAAACGCCAGGCCAGGCAGCACTAA
AGGTTAAAGGTCAAATTGGAAGCTAAGGCTATAAATCATCCTGGGTTCCAGGCTTA
AATCTTGCAATGGACACTCTCCCCAACCATAAAGCCTTAGCTCTGGTCTCCATGG
AATCATGCAGGTCAACATAAAACTGGATTCTGGACTGCGTGGCTAAAGCACTT
AGACTARGAGTCCAGTGTGACTGGATGGATAGGGCCTCAGCTGTCAACTCTAA
GTTAGMGMTCATGGAATGAAGGCCTGRGGGCTGCTCAAGTCTGTTAGGTTCTG
CTTGGAAAGATGACCACCTGGAGGTGGCCGGCCTTTGGTTGGCTGGTTGT
GTTATAGACACAAGCCTATGGAAAGGAACCGTCTGGCTTAAAGAAATTACTATG
TTCCTGGAGTTGGTGGTAACCAGCTGCTTGCAGATGATGGGTGAAGTGGAAAGG
GATGGCTTGTGAGGCTGACCAAGTCTGTACCGGATGTTGTACAGATTCCCTCCC
ACACCGGAGACATCGTACTATATTAGAAACAGCCACGGACTTGTGCTTTAGTT
TGTGTCCTGGAAACATACGGGGGGCAGGCTGTTGCTGGTACCTGGGGCCCTGC
CCTCCCAGACACGGGAGTGCTGTAGCGTGGAGGGCCAGTTGGCCAGATTGTTA
GCTCTGCGTTGGGTGTCGTAGACAACGTACAGGATTAGCCTAACCCAAGCACT
GAGTGAGGTGATTTCCCTGGCTTTGGCGTGTCTTGGTATTACCATGTATTGT
GGTGTAGGTAGTGTGTCAGGTACTGTTGGCTGTGTCTCCTAGACTAAGCGGGCGTT
GSATACAGCTTACATACAGTGCCTGGAGACCAAGGTAGTTGGTTGTAATAAGCTG
GTCCACCCTAACAGACTCCAAACATYACAGAAGCTYTTATGGMCCTACCTAAT

AATGCCAATTCTGGAGGACACTCTTTACCATAAGAWKCSAATCCTGATTCCTGGC
TCCTGGTTGAGCTTCCGCACGTACATACACCCCTCTGRCTGCCATCAGGGCATTGCT
GCTGAGTTCTGCATTGCTTAACCTSCKGSYGYTTCTGCCTAAAGGGATGCCACCC
AGACACCTAAAAAGACCCGGATGGCTCTAGCCTGGTGGAGAGTCTTATTAGAA
5 GTTTCTTGGGGATTGGGATTGGCTCAGTGGTAGAGCGCTGCCTGGCAAGCA
CAAGGCCCTGGGTCGGTCCCCAGCTCTAAAAAAAAAAAAAGTTTCTTGGTAGT
TGGGAAAAGGCAGAAGGAAAAACAAAGGGAAAGATGAATCTCTCAGCCTAC
CTGGTCCCTAAATTAAATCGTGTATGTGACTAGTTAAGTCTCTTGACTAACAA
AGGGACACCAGGTTCTGGGAGAAATCTCAGAGCAAATGTTGCCTGTTGSTAAC
10 TTCTGGTAACCARAGGARCCCTGATAARCTTARGAGYKGACTGTATGTCCATGCTCT
TGTGACTCTAGAGACTCTGGCACCTCAGGTNAAGCAGGCTGTGAGCCAGATGTCCT
GGTCCAAGCAACCCACTGTTGAGCAGCAGGGCACCATAGGCCTCAGCTAGGGG
AGCGCACTGGTAGAGCCAGCAAGTGAGCAGGAATCTGACTTAGGGTAAAAATCTA
GACAGTTCTGACAGCTGGAAGTCAACTTTCCATTCAAAGTCATGTGGCATTGG
GAAGGGCTAGGAAATAGAAGTGGGTTCCAGCTTATCTCCTACACAGTCTCGAG
TATAGCATTAACACCGAGTGCTGGACAGAGGTTGCTGCTGAACACTCAATCCTGCT
CCTGACTGACTCTGGAAATAAGGACATTCCACTCTGCTTGGCGCGAGATGCCCTAG
TGTGCGGCCGCGGGGCTCTCTCAAGTCCTACAGNACTCCAGGCAGTTC
ATCTCCTAGGAAAAGGTATGGAGGTTGCTGCTTACGGTAAACACAGGATAAA
ATCTACAGTAAACAACCGGTAAAGTGCTGGCTTACGCCCTGGCTTCTCCAGGCA
20 CAGGTGGGTTGACTACTCCCATTCTCATTTGTAAGCACCTCAGGTTAGGGCAG
TTCTTCAGAGTTGGGGGACTGGAGCCATTCCCTGTAATGCCTGAGGTGGCCTT
ACCACCTAGCAGCCAGTTGCCAGCAACAGCCACACTGCTGTTAGGTATCATAAT
ACCTCATCCTCGGGTTCTCAGAAAGGRAAAWGCTAACTCAGTTGATGTAAGTGT
25 TGCTGTGCTGGATCCTGTCATGTGGAGGGAACACCAAATACACAGGCTCTCAGG
AGACATCTGCTAAGGCTCTTACTGCAGTCTGCTCACGTTGAAATCTGCCCTC
TGTCTCCTGACTCARAAAGACTCAGCCMCAAATCAAGAAGCGCCATCAAACGTTCC
TTCTCAKKGGAACGTGCTCCACAGGAAGGTCCAGWGGGATTGCARCTAGAGTCA

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CGTTTACTGGKTTGTGAMCAAATTACTGGTTTCARTTACCTGGGGKCCTATGKG
KKTTTMAACCTTCCCATMAGGCAGTTAGTAGCCACTTGGGTTCTGTGGA
CGTGCCTCAGCTCTCGGCATAGGAACCCAACAGGTAGAATACTGAAACTTCTCAG
TGGCCAAGACCTCGATACCCCTCTGATGGGTGGAACTGGCTATTCCTGACCA
5 ATCTAGGCCACCATTAGTCCCTGGTCACATTCTACTCCAAACTGAAATTCAAGTT
TGGCTTGAGTATGTGCACACGTGGTGGGTCACCTACTTCAGTGTGACCAAAAGT
TTATTTCTAGTGCATTCTAAATGGTAAAATATGTAATTAGTATGCATGAC
TGGGTCTCCAAAATAAAACTGAGTGTATTGTGAAAAAAAAAAAAAAA
AAAA-3' (SEQ ID NO:58). The following nucleic acid sequence is the ORF for rat R286: 5'-
10 ATGTCAGAAGAAAGTGACTCTGTGAGAACCAAGCCCCCTGTGGCCTCACTCTC
CGAAAATGAGCTGCCACCGCCTCCCCGGAACCTCCCGCTACGTGTGCTCGCTGAC
AGAAGACTTGGTCACCAAGGCCAGGGAAAGAGCTTCAGGAGAACGCCAGTGGAGAC
TCCGGGATGTGCAGGCCCTCGAGACATGGTACGGAAGGAGTACCCATACCTGAGT
ACATCGCTGGATGATGCCTCCTGTTGCGCTTCTGAGGGCCCGAAAGTTGATTATG
ACCGGGCCCTGCAGCTGGTCAACTACCATGGCTGCAGGCCAGTGGCCAGAG
GTCTTCAGCAACCTGAGGCCATGCCCTGAAAGACGTTCTTAACCTGGATTCTC
ACAGTGCTGCCAACACAGACCCAGGGCTGCCATGTCCTCTGCATCCGACCAGAC
AGATGGATACCGAGCAACTACCCGATCACCAGAGAACATCCGCGCCATCTACTGAC
GTTAGAAAAACTCATTCACTGGAGGAGACCCAGGTGAACGGGTTGTAATCCTCG
CCGACTACAAGGGAGTGAGCTTATCAAAGGCGTCTCACTTGGCCCTTATGCCA
20 GAAAGGTGATTGGCATCCTCAGGATGGCTCCATTGGATAAAAGCAGTCACA
TAGTAAACGAACCTCGGATATTAAAGGGATTTCGCCATCATAAAACCATTCTGA
AGGAGAAAATTGCAAACAGGTTCTCCTCCATGGGTCTGACCTGAGCTCTGCACA
CGAGCCTCCAAGGAATATCCTCCCCAAAGAGTATGGGGCACCGCTGGGAGCTG
25 GACACTGCCAGCTGGAACCGCGGTGCTGGCCTCGGAGGATGATTGTGAAAGA
GTTCTGCCAGCCTGAGTCTGGCTCGATGGCTCTTGGGCCAGCCCTGCTGCCTGA
GGGGCTGATCTCAGACGCGCAGTGTGACGACTCCATGCGAGCCATGAAGTCCCAGC
TCTACTCCTGCTATTAG-3' (SEQ ID NO:59). Using the rat R286 cDNA sequence and a

portion of the human R286 nucleic acid sequence, specific primers were designed to amplify the human R286 homologue. After RT-PCR using human hippocampal RNA and the specific primers, the PCR product was subcloned in the TA-cloning vector (InVitrogen) and sequenced with SP6 and T7 primers. The following nucleic acid sequence is the ORF for human R286: 5'-

5 ATGTCCGAAGAAAGGGACTCTCTGAGAACCGAGCCCTCTGTGGCCTCACTCTTGAA
AATGAGCTGCCACCACCTGAGCCTCCGGCTATGTGTGCTCACTGACAGAAGAC
CTGGTCACCAAAGCCCAGGAAGAGCTGCAGGAAAAGCCGAATGGAGACTTCGAGA
TGTGCAGGCCCTCGTGACATGGTGCAGAAGGAGTACCCAACCTGAGCACATCCCT
CGACGATGCCTCCTGCTCGCTCCCTCCGAGCCCGCAAGTTGATTACGACCGGGC
10 CCTGCAGCTCCTCGTCAACTACCACAGCTGTAGAAGAAGCTGGCCGAAGTCTCAA
TAACCTGAAGCCATCAGCCTAAAAGATGTCCTGCTTCCGGTTCTCACCGTGCTG
CCCCACACTGACCCCAGGGCTGCCATGTCGTCATCCGCCAGACAGATGGATA
CCAAGCAACTATCCAATTACTGAAAACATCCGAGCCATATACTTGACCTTAGAAAAA
CTCATTCACTGAAGAAACCCAGGTGAATGGAATTGTAATTCTGCAGACTACAAA
GGAGTGAGTTATCAAAAGCATCTCACTTGGCCCTTTATAGCCAAAAGGTGATT
GGCATCCTCCAGGATGGTTCCCCATTGGATAAAAGCAGTCCATGTGGTGAATGAA
CCTCGAATATTAAAGGCATTTGCCATCATAAAACCATTCTAAAGGAGAAAATA
GCAAACAGATTCTCCTCCATGGGTCTGACTGAACCTCTCCACACAAACCTCCA
AGAAGCATCCTCCCCAAGGAGTATGGGGCACGGCTGGGAGCTGGACACTGCCAC
CTGGAACGCAGTACTGCTGGCTTCAGAAGACGATTGTGAAAGAGTTCTGCCAAC
TGTTCCTGCCTGTGACAGCATCCTGGCCAGACGCTGCTGCCAGGGCCTGACCTC
AGATGCACAGTGTGACGACTCCTGCGAGCTGTGAAGTCACAGCTGTACTCCTGCTA
CTAG-3' (SEQ ID NO:60). The R286 clones were found to be homologous to a family of transfer proteins for hydrophobic ligands (such as lipid soluble vitamins and phospholipids).

25 Thus, R286 is a lipid transfer polypeptide. The amino acid sequence of the rat R286 polypeptide is as follows: MSEESDSVRTSPSVASLSENELPPPPPEPPXYVCSDLTEDLVTKAREEL
QEKPFWRLRDVQALRDMVRKEYPYLSTSLLDAFLLRFLRARKFDYDRAQLLVLNYHGC
RRSWPEVFSNLRPSALKDVLSNGFLTVLPHTDPRGCHVLCIRPDRWIPSNYPITENIRAIY

10 LTLEKLIQSEETQVNGVVILADYKGVSLSKASHFGPFIARKVIGILQDGPIRIKAVHIVNE

5 PRIFKGIFAIKPFLKEKIANRFFLHGSDLSSLHTSLPRNILPKEYGGTAGELDTASWNAVL

LASEDDFVKEFCQPESGCDGLGQPLLPEGLISDAQCDDSMRAMKSQLYSCY (SEQ ID
NO:61). The amino acid sequence of the human R286 polypeptide is as follows: MSEERDSL

RTSPSVASLSENELPPPPEPPGYVCSLTEDLVTKAREELQEKPWEWRLRDVQALRDMVRKE

YNPLSTSLLDAFLLRFLRARKFDYDRALQLLVNYHSCRRSWPEVFNNLKPSALKDVLAS

GFLTVLPHTDPRGCHVVCIRPDRWIPSNYPITENIRAIYLTLLEKLIQSEETQVNGIVILADY

KGVSLSKASHFGPFIAKKVIGILQDGPIRIKAVHVNEPRIFKGIFAIKPFLKEKIANRFFL

HGSIDLNSLHTNLPRSILPKEYGGTAGELDTATWNAVLLASEDDFVKEFCQPVPACDSILG

15 QTLLPEGLTSDAQCDDSLRAVKSQLYSCY (SEQ ID NO:62).

Northern blot and *in situ* analysis using a sequence from the R286 clone as a probe revealed the presence R286 mRNA throughout rat brain. For *in situ* hybridization, Dig-labeled cRNA probes were used as described elsewhere (Kuner et al., *Science* 283:5398 (1999)). Specifically, R286 mRNA expression was the highest in the cortex and hippocampus while being moderately high in the cerebellar granule cells, brainstem nuclei, several lateral and medial thalamic nuclei, olfactory bulb, and striatum. In addition, this analysis revealed that the expression of the R286 mRNA was upregulated in response to the multiple MECS treatment. Briefly, a probe from the 3' untranslated region of R286 was used to hybridize a Northern blot containing 2 µg polyA⁺ RNA from hippocampus from brains of untreated rats as well as rats receiving the multiple MECS treatment. After one day of exposure using the phosphoimager FLA2000 (Fuji), an upregulation of R286 mRNA was detected in the hippocampus (3.72 fold induction) collected four hours after the last MECS treatment. An additional Northern blot analysis using 10 µg total RNA from hippocampus from untreated rats and rats receiving the multiple MECS treatment was performed. In this experiment, the probe was the ORF of R286 and the level of expression was found to be induced 2.4 fold in the MECS treated animals (Table I).

In addition, rats that developed seizures following intraperitoneal injection of kainate or PTZ were analyzed for the expression of R286 mRNA in addition to the mRNA of other IEG

clones (Tables III and V). R286 mRNA expression was observed, by *in situ* hybridization, to be mildly upregulated in the hippocampal pyramidal cell layer, cortex, thalamus, and cerebellar Purkinje cell layer at 6 hours post-kainate injection. At 6 hours post-PZT injection, R286 mRNA expression was observed to be mildly upregulated in these brain structures, while no upregulation was observed at 20 minutes post-PTZ injection or at 1.5 hours post-kainate injection.

Other IEG nucleic acid clones included L073 (concatamer with Krox-20), L125 (oxoglutarate carrier protein), L201 (concatamer), R094 (fra2), and R217 (diacylglycerol kinase; accession #D78588).

10 OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

- 1 1. An isolated nucleic acid comprising at least one adenine base, at least one guanine base,
2 at least one cytosine base, and at least one thymine or uracil base, wherein said isolated nucleic
3 acid is at least 12 bases in length, and hybridizes to the sense or antisense strand of a second
4 nucleic acid under hybridization conditions, said second nucleic acid having a sequence as set
5 forth in SEQ ID NO:1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 22, 23, 24, 25, 28, 29, 33, 34,
6 35, 37, 39, 40, 41, 42, 43, 44, 47, 49, 50, 51, 52, 53, 54, 55, 56, or 57.
- 1 2. The isolated nucleic acid of claim 1, wherein said hybridization conditions are
2 moderately stringent hybridization conditions.
3. The isolated nucleic acid of claim 1, wherein said hybridization conditions are highly
stringent hybridization conditions.
4. An isolated nucleic acid, wherein said isolated nucleic acid comprises a nucleic acid
sequence that encodes an amino acid sequence at least five amino acids in length, said amino
acid sequence comprising at least three different amino acid residues, and being identical to a
contiguous portion of sequence set forth in SEQ ID NO:11, 21, 30, 36, 38, or 48.
- 1 5. An isolated nucleic acid comprising a nucleic acid sequence at least 60 percent identical
2 to the sequence set forth in SEQ ID NO:1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 22, 23, 24,
3 25, 28, 29, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 47, 49, 50, 51, 52, 53, 54, 55, 56, or 57.
- 1 6. An isolated nucleic acid, wherein said isolated nucleic acid comprises a nucleic acid
2 sequence that encodes an amino acid sequence at least 60 percent identical to the sequence set
3 forth in SEQ ID NO:11, 21, 30, 36, 38, or 48.

1 7 An isolated nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO:1,
2 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 22, 23, 24, 25, 28, 29, 33, 34, 35, 37, 39, 40, 41, 42, 43,
3 44, 47, 49, 50, 51, 52, 53, 54, 55, 56, or 57.

1 8. A substantially pure polypeptide comprising an amino acid sequence encoded by a
2 nucleic acid of claim 1.

1 9. A substantially pure polypeptide comprising an amino acid sequence as set forth in SEQ
2 ID NO:11, 21, 30, 36, 38, or 48.

1 10. A substantially pure polypeptide comprising an amino acid sequence at least 60 percent
2 identical to the sequence set forth in SEQ ID NO:11, 21, 30, 36, 38, or 48.

1 11. A substantially pure polypeptide comprising an amino acid sequence at least five amino
2 acids in length, said amino acid sequence comprising at least three different amino acid residues,
3 and being identical to a contiguous stretch of sequence set forth in SEQ ID NO:11, 21, 30, 36,
4 38, or 48.

1 12. A host cell containing an isolated nucleic acid of claim 1.

1 13. The host cell of claim 12, wherein said host cell is a eukaryotic cell.

1 14. An antibody having specific binding affinity for an amino acid sequence encoded by a
2 nucleic acid of claim 1.

1 15. The antibody of claim 14, wherein said antibody is monoclonal.

1 16. The antibody of claim 14, wherein said antibody is polyclonal.

- 1 17. A cDNA library comprising a plurality of clones, wherein each clone comprises a cDNA
2 insert and wherein at least about 15 percent of said clones comprise cDNA derived from
3 immediate early genes.
- 1 18. The cDNA library of claim 17, wherein at least about 20 percent of said clones comprise
2 cDNA derived from immediate early genes.
- 1 19. The cDNA library of claim 17, wherein at least about 25 percent of said clones comprise
2 cDNA derived from immediate early genes.
- 1 20. The cDNA library of claim 17, wherein said immediate early genes are immediate early
2 genes responsive to a maximal electroconvulsive seizure.
21. The cDNA library of claim 17, wherein said cDNA library is a subtracted cDNA library.
22. The cDNA library of claim 21, wherein said subtracted cDNA library is IEG-Reg cDNA
library.
23. The cDNA library of claim 21, wherein said subtracted cDNA library is IEG-Lg cDNA
library.
- 1 24. An isolated nucleic acid derived from a cDNA library, wherein said cDNA library
2 comprises a plurality of clones, wherein each clone comprises a cDNA insert and wherein at
3 least about 15 percent of said clones comprise cDNA derived from immediate early genes.
- 1 25. The isolated nucleic acid of claim 24, wherein said isolated nucleic acid comprises a
2 nucleic acid sequence of an immediate early gene.

1 26. A method of obtaining immediate early gene nucleic acid, said method comprising:
2 a) providing a cDNA library, said cDNA library comprising a plurality of clones,
3 wherein each clone comprises a cDNA insert and wherein at least about 15 percent of said clones
4 comprise cDNA derived from immediate early genes;
5 b) contacting at least a portion of said cDNA library with a probe, said probe containing
6 at least one nucleic acid having a nucleic acid sequence derived from an immediate early gene;
7 and
8 c) selecting a member of said plurality of clones based on the hybridization of said at
9 least one nucleic acid to said member under hybridization conditions, said member comprising
10 said immediate early gene nucleic acid.

27. A method of treating an animal having a deficiency in a neuron's immediate early gene responsiveness to a stimulus, said method comprising administering a nucleic acid of claim 1 to said animal such that the effect of said deficiency is minimized.

28. The method of claim 27, wherein said deficiency comprises a reduced level of expression of an immediate early gene.

29. The method of claim 27, wherein said stimulus influences learning or memory.

1 30. The method of claim 29, wherein said stimulus comprises a maximal electroconvulsive
2 seizure.

1 31. A method of treating an animal having a deficiency in a neuron's immediate early gene
2 responsiveness to a stimulus, said method comprising administering a therapeutically effective
3 amount of a substantially pure polypeptide of claim 8 to said animal such that the effect of said
4 deficiency is minimized.

1 32. A method of treating an animal having a deficiency in a neuron's immediate early gene
2 responsiveness to a stimulus, said method comprising administering an effective amount of cells
3 to said animal such that the effect of said deficiency is minimized, said cells containing a nucleic
4 acid of claim 1.

1 33. A method of treating an animal having a deficiency in a neuron's immediate early gene
2 responsiveness to a stimulus, said method comprising administering a therapeutically effective of
3 antibodies to said animal such that the effect of said deficiency is minimized, said antibodies
4 having specific binding affinity for an amino acid sequence encoded by a nucleic acid of claim 1.

1 34. The method of claim 33, wherein said deficiency comprises an elevated level of
2 expression of an immediate early gene.

3 35. A method of identifying a compound that modulates immediate early gene expression,
4 said method comprising:
5 a) contacting a test compound with an immediate early gene nucleic acid; and
6 b) determining whether said test compound effects the expression of said immediate early
7 gene nucleic acid, wherein the presence of an effect indicates that said test compound is said
8 compound.

1 36. The method of claim 35, wherein said immediate early gene nucleic acid comprises a
2 nucleic acid sequence as set forth in SEQ ID NO:1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 22,
3 23, 24, 25, 28, 29, 33, 34, 35, 37, 39, 40, 41, 42, 43, 44, 47, 49, 50, 51, 52, 53, 54, 55, 56, or 57.

1 37. The method of claim 35, wherein said effect is a reduction in the expression of said
2 immediate early gene nucleic acid.

1 38. The method of claim 35, wherein said effect is an increase in the expression of said
2 immediate early gene nucleic acid.

1 39. A method of identifying a compound that modulates immediate early gene polypeptide
2 activity, said method comprising:

3 a) contacting a test compound with an immediate early gene polypeptide; and
4 b) determining whether said test compound effects the activity of said immediate early
5 gene polypeptide, wherein the presence of an effect indicates that said test compound is said
6 compound.

1 40. The method of claim 39, wherein said immediate early gene polypeptide comprises an
2 amino acid sequence encoded by a nucleic acid of claim 1.

1 41. The method of claim 39, wherein said immediate early gene polypeptide comprises an
2 amino acid sequence as set forth in SEQ ID NO:11, 21, 30, 36, 38, or 48.

1 42. The method of claim 39, wherein said effect is a reduction in the activity of said
2 immediate early gene polypeptide.

1 43. The method of claim 39, wherein said effect is an increase in the activity of said
2 immediate early gene polypeptide.

ABSTRACT

The present invention provides methods and materials related to immediate early genes. Specifically, the invention provides isolated immediate early gene nucleic acid, cells that contain isolated immediate early gene nucleic acid, substantially pure polypeptides encoded by immediate early gene nucleic acid, and antibodies having specific binding affinity for a polypeptide encoded by immediate early gene nucleic acid. In addition, the invention provides cDNA libraries enriched for immediate early genes cDNAs, isolated nucleic acid derived from such cDNA libraries, and methods for treating conditions related to a deficiency in a neuron's immediate early gene responsiveness to a stimulus.

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COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled IMMEDIATE EARLY GENES AND METHODS OF USE THEREFOR, the specification of which

- is attached hereto.
 was filed on February 5, 1999 as Application Serial No. _____
and was amended on _____.
 was described and claimed in PCT International Application No. _____
filed on _____ and as amended under
PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. SERIAL NO.	FILING DATE	STATUS
60/074,518	2/12/98	<input checked="" type="checkbox"/> Pending <input type="checkbox"/> Issued <input type="checkbox"/> Abandoned
60/074,135	2/6/98	<input checked="" type="checkbox"/> Pending <input type="checkbox"/> Issued <input type="checkbox"/> Abandoned

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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